EDITORIAL BOARD

Editorial office address: 656038, RF, Altai Krai, Barnaul, Lenina Prospekt, 40, office 220
Tel.: +7(3852) 566869. E-mail: bmn@agmu.ru. www.bmn.asmu.ru
Registration certificate SMI PI № FS 77 – 69379 from 6th of April 2017, issued by the Federal Service for Supervision of Communications, Information Technology, and Mass Media
Russian version ISSN 2541-8475
English version ISSN 2542-1336
Founder and publisher
Federal State Budgetary Educational Institution of Higher Education "Altai State Medical University" of the Ministry of Health of the Russian Federation (FSBEI HE ASMU of the Ministry of Health of the Russian Federation), 656038, RF, Altai Krai, Barnaul, Lenina Prospekt, 40
www.asmu.ru

Print. LLC "AZBUKA". RF, Altai Krai, Barnaul, Merzlikina Street, 10.
Format: 60x90 1/8. Conventional printed sheets – 8,4. Circulation – 500 copies. Open price.

CONTENT

THE ASSESSMENT OF RISK OF CHEMICAL AIR POLLUTION FOR THE POPULATION HEALTH ACCORDING TO THE RESULTS OF SOCIAL-HYGIENIC MONITORING IN ALTAI KRAI
I.G. Pashchenko, A.A. Ushakov, A.S. Katunina 3

RISK STRATIFICATION OF INFECTIONS CONNECTED WITH HEALTHCARE DELIVERY IN PATIENTS WITH MALIGNANT NEOPLASMS ON THE BACKGROUND OF CHEMOTHERAPEUTIC AND RADIOTHERAPEUTIC TREATMENT IN ALTAI KRAI
A.N. Neshatayev, N.V. Lukyanenko , A.F. Lazarev, N.Ya. Lukyanenko..... 6

ASSESSMENT OF EATING BEHAVIOR IN PUPILS OF MOSCOW AND ALTAI KRAI AND THE ROLE OF FAMILY AND SCHOOL IN THE FOOD CULTURE FORMATION
Zh.Yu. Gorelova, S.P. Filippova, Yu.V. Solovyeva, A.N. Plats-Koldobenko, T.A. Letuchaya, I.P. Saldan, O.V. Zhukova 10

THE ASSESSMENT OF RISK OF DRINKABLE WATER CHEMICAL POLLUTION TO THE HEALTH OF ALTAI KRAI POPULATION
T.I. Gubareva, A. Yu. La, Yu.A. Kapayeva, L.A. Skripkina, N.Ye. Soldatova..... 14

CURRENT EPIDEMIOLOGICAL SITUATION ON BACTERIAL MENINGITIS IN ALTAI KRAI
Yu.A. Kozyanova, T.V. Safyanova, S.V. Shabanova..... 17

PHARMACOLOGICAL MODULATION OF THE EXPRESSION OF INTRARENAL INHIBITORS OF CRYSTALLIZATION AND OXIDATIVE STRESS MARKERS OF NEPHROCYTES BY EXPERIMENTAL KIDNEY STONE DISEASE
A.Yu. Zharikov, N.N. Yakushev 20

COMPARATIVE ASSESSMENT OF HISTOCHROME'S ANTIOXIDANT AND PROOXIDANT ACTIVITIES IN TERMS OF ***IN VITRO*** AND ***IN VIVO*** EXPERIMENTS
O.S. Talalayeva, Ya.F. Zverev, S.V. Zamyatina 24

POTENTIAL FOR USE OF FOMES OFFICINALIS EXTRACT IN THERAPY OF TOXIC HEPATITIS
O.N. Mazko, O.G. Makarova, L.G. Dvornikova, A.P. Pashkov 29

STUDY OF THE POLYSACCHARID COMPLEX LAVATERA THURINGIACA L., GROWING IN ALTAI KRAI
L.M. Fedoseyeva, O.A.Myznikova 32

ADAPTOGENIC EFFECT OF PRODUCTS OF ANTLER STAG BREEDING ON THE STATE OF HEMOSTATIC SYSTEM IN RATS BY SUPRATHRESHOLD PHYSICAL LOAD
A.A. Blazhko, I.I. Shakhmatov, V.I. Kiselev, A.Yu. Zharikov 36

SUSTAINABLE THERAPY OF UROGENITAL cHlamYdiosis
I.O. Malova, I.A. Sidorova..... 40

CLINICAL SUPERVISION OF THE EFFICIENCY OF THE UNIVERSAL HYGIENIC MEDICATION BY SKIN LESIONS
N.V. Shperling, I.A. Shperling..... 46

LABORATORY DIAGNOSTICS OF TISSUE INFECTIONS WITH NATURAL FOCUS (TICK-BORNE RICKETTSIOSIS, IXODIC TICK-BORNE BORRELIOSIS)
O.V. Beskhlebova, V.M. Granitov, V.G. Dedkov 50

ENZYME PREPARATION IN THERAPY FOR LICHEN SCLEROSIS IN THE FEMALE EXTERNAL GENITAL ORGANS
Yu.S. Kondratyeva, S.O. Filinova 55

OPTIMIZATION OF PROSTHETIC FITTING OF FULL-CERAMIC RESTORATIONS IN DENTISTRY
Grokhotov I. O, Ubrayev R. Ye...... 59

OBSTETRICAL ASPECTS OF FORMATION OF HYPOXIC-ISCHEMIC CNS DISORDERS IN FULL-TERM LOW-WEIGHT NEWBORNS
N.S. Dolgova, S.D. Yavorskaya, L.P. Ananyina, Ye.V. Pozhenko 62

ENDOMETRIUM RECEPTION AND SOME IMMUNO-HISTOCHEMICAL PARAMETERS BY CHRONIC ENDOMETRITIS ON THE BACKGROUND OF TREATMENT BY THE EXTRACT OF ORTILIA SECUNDA
O.A. Skoropatskaya, N.I. Fadeyeva, S.D. Yavorskaya, T.S. Taranina..... 65

ALTERATIONS OF THE HEMOSTATIC SYSTEM IN THE IVF CYCLE AND THEIR INFLUENCE ON THE PROCEDURE EFFICIENCY
A.P.Momot, I.V. Molchanova, L.P. Tsyvkina..... 68

Requirements for publication in the «Bulletin of Medical Science» Journal..... 73

UDC 613.31:543.3:351.77 (571.15)

THE ASSESSMENT OF RISK OF CHEMICAL AIR POLLUTION FOR THE POPULATION HEALTH ACCORDING TO THE RESULTS OF SOCIAL-HYGIENIC MONITORING IN ALTAI KRAI

Directorate of the Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing for Altai Krai, Barnaul

I.G. Pashchenko, A.A. Ushakov, A.S. Katunina

The article presents the data on the health status, sanitary-hygienic conditions of living of the Altai Krai population. According to the laboratory tests conducted in terms of social-hygienic monitoring: there was performed the analysis of data on the state of air in populated areas of the region; there were determined leading chemical substances polluting the air in populated areas of the region, which could have influenced the health of population; there was given the characteristic and evaluation of risk for the population health.

Key words: *health status, air of populated areas, population under influence, chemical substances, cancerogenic, noncancerogenic risk for population health.*

One of the key objectives of social-hygienic monitoring (further – SHM) as the federal system of surveillance, analysis, assessment and prognosis of health status of the population and human environment is the determination of causal connection between the health status of the population and the influence of human environment factors on the basis of systemic analysis and evaluation of population health risk [1].

The implementation of methods of health risk evaluation allows: to obtain quantitative characteristics of health damage by environmental hazards; to compare and range effects of human environment factors different in the degree of influence; to identify sensitive and vulnerable subgroups of population most exposed to unfavorable influence [2].

The influence of environmental harmful factors leads to the reduction of nonspecific resistance, occurrence of various types of diseases, determining complex processes of long-term interaction of exogenous and endogenous factors [5,4].

The contamination of atmosphere on the territory of populated areas contributes to the growth of number of children with allergic and respiratory diseases. The effect of the most spread air pollutant - carbon oxide on human organism cells manifests itself through disorders of tissue respiration processes, reduction of oxygen tissue consumption [7]. By long-term inhalation of sulphur dioxide, the first symptoms of intoxication are vegetovascular dysfunction, neurocirculatory disorders in combination with stomach and liver damage. It is known, that the increase of daily average concentration of sulphur dioxide by every 10 mkg/m3 leads to the growth of overall mortality by o,6%, mortality of repiratory diseases – by 1,2%; mortality of cardiovascular diseases – by o,6% [3, 6]. According to WHO, by the increase of nitrogen dioxide concentration in the air by 30 mkg/m3, the number of children at the age of 5-12 with lower respiratory tract diseases grows by 20%. By incomplete combustion of mineral fuel there forms black carbon – particles of solid carbon, on the surface of which there is adsorbed benzapyrene, formaldehyde, benzol. Entering the human organism, these substances can cause malignant tumor formation.

Research objective

The hygienic analysis of sanitary-hygienic human environment factors of Altai Krai population (further – region), in particular, the air of populated areas, determining the leading chemical substances polluting the air and evaluation of risk for the population health. Research targets were municipal units of the region (60 rural and 10 urban) characterized by the indexes of social and economic development, health status, hygienic state of environment for the period of 2014-2016 (further long-term average annual period).

Materials and methods

Research materials were databases of the federal and regional funds of SGM data (further – FIF and RIF SGM) of the Directorate of the Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing in Altai Krai (further – Directorate) for the period of 2014-2016 on hygienic environmental factors of human environment: air at the points of surveillance in populated areas of the region by concentration of 26 polluting substances; 98 957 tests of air in populated areas; data on the protection of enterprise air (form № 2-TP (air) and MPE volumes); health status of children at the age of 0-14, teenagers at the age of 15-17, adults at the age of 18 and older.

The data analysis was performed by means of hygienic and statistical methods, methods of risk assessment [2]. The statistical data processing was

conducted by means of Statistica 6.0 computer program and Excele applications.

The air examination was performed in 24 populated localities. The study was conducted at 44 points of air surveillance, including: fixed monitoring stations of the complex laboratory of environmental pollution monitoring and route monitoring stations of the FBHI Center of Hygiene and Epidemiology in Altai Krai (further – FBHI CHE in Altai Krai) and its affiliated brances.

Priority substances were chosen on the basis of data on the protection of atmospheric air of enterprises (form № 2-TP (air) and MPE volumes). There was determined the list of priority substances able to influence the population health: Sulphur dioxide, nitrogen (IV) oxide, manganese and its compounds, cupric (II) oxide, benzene (petroleum, low-sulphur) / in terms of carbon content/, naphthalene, hydrogen sulphide, pyridine, dipping acid, hydrocyanite, prop-2-en-1-al, carbon black, xylol, nitrogen (II) oxide, iron (II) oxide, barium and its salts, carbon oxide, formaldehyde, lead and its inorganic compounds, inorganic dust containing silicone dioxide under 20%, carbolic acid, vanadium pentoxide (dust), hydrochloride, coal ash of thermal power plants, chrome (+6), chlorine, benzol, ammonia, nitrogen (IV) oxide, benzapyrene.

There was made the identification risk of hazardous substances emissions into the air, there were calculated the coefficients of cancerogenic hazard and indexes of relative hazard of noncancerogenic effect, which allowed to range the substances.

There was assessed the risk of noncancerogenic effect of chronic influence on the population. There was determined the expectancy of development of health detrimental effects – the expected number of annual additional cases of diseases (per 1 thousand population) in 11 cities and 13 rural units of the region.

Results and discussion

Out of 24 populated locations, where there was assessed the cancerogenic risk, there were determined: 8 locations with ***maximum permitted level of cancerogenic risk*** for population's health (over 1 x 10^{-6}, but under 1 x 10^{-4}); 13 locations with ***average level of cancerogenic risk*** for population's health (over 1 x 10^{-4}, but under 1 x 10^{-3}); 1 location with ***high level of cancerogenic risk*** (equal or over 1 x 10^{-3}); 2 locations with ***non-controlled cancerogenic pollutants*** of the atmospheric air.

The change of human environment quality leads to the reduction of quality of life, as is evident from medical and demographic indexes and given levels of incidence.

There is registered the exceedance of average regional indexes of *primary disease incidence* for the period of 2014-2016 (per 10 000 population) among:

1. Among the *whole population* (over 13 788,5$^0/_{000}$) – in 3 cities and 15 rural administrative districts.

2. According to the class of *respiratory diseases* among the *whole population* (over 3 757,1$^0/_{000}$) – in 3 cities and 14 rural administrative districts, including nosological forms: *chronic and unspecified bronchitis, emphysema* (50,2$^0/_{000}$) in 2 cities and 20 rural administrative districts; *asthma, status asthmaticus* (12,3$^0/_{000}$) in 4 cities and 32 rural administrative districts.

3. Among *children* (from 0 to 14 years inclusively) (over 18 125,2$^0/_{000}$) – in 4 cities and 12 rural administrative districts.

4. According to the class of *respiratory diseases* among *children* (from 0 to 14 years) (over 11 848,0$^0/_{000}$) – in 3 cities and 8 rural administrative districts, including nosological forms: *chronic and unspecified bronchitis, emphysema* (over 0,4$^0/_{000}$) in 1 city and 8 rural administrative districts; *asthma, status asthmaticus* (over 15,4$^0/_{000}$) in 4 cities and 8 rural administrative districts.

5. Among *teenagers* (from 15 to 17 years inclusively) (over 18 461,1$^0/_{000}$) – in 3 cities and 19 rural administrative districts.

6. According to the class of *respiratory diseases* among *teenagers* (from 15 to 17 years inclusively) (over 8 812,0$^0/_{000}$) – in 3 cities and 22 rural administrative districts, including nosological forms: *chronic and unspecified bronchitis, emphysema* (over 7,8$^0/_{000}$) – in 1 city and 6 rural administrative districts; *asthma, status asthmaticus* (over 12,5$^0/_{000}$) in 4 cities and 12 rural administrative districts.

7. Among *adults* (from 18 years and older) (over 9 229,0$^0/_{000}$) – in 2 cities and 7 rural administrative districts.

8. According to the class of *respiratory diseases* among *adults* (from 18 years and older) (over 1 830,4$^0/_{000}$) – in 2 cities and 11 rural administrative districts, including nosological forms: *chronic and unspecified bronchitis, emphysema* (over 62,4$^0/_{000}$) – in 2 cities and 20 rural administrative districts; *asthma, status asthmaticus* (over 11,6$^0/_{000}$) in 3 cities and 38 rural administrative districts.

There was also stated the exceedance of average regional indexes in 2016:

1. *For the first time in life stated diagnosis of malignant neoplasms* among the *whole population* (over 43,5 per 10 000 population) is registered in 5 cities and 29 rural administrative districts (in 2015 - – 44,9$^0/_{000}$; 2014 – 42,2$^0/_{000}$).

2. *For the first time in life stated diagnosis of malignant neoplasms of trachea, bronchi, lungs* among the *whole population* (over 5,2 per 10 000 population) – in 3 cities and 36 rural administrative districts (2015 – 5,5$^0/_{000}$; 2014 – 4,9$^0/_{000}$).

Uncertainties by risk evaluation. Uncertainty – is a situation determined by the imperfection of knowledge about the parameters and processes used for the risk assessment [1]. In the current study, at the stage of identification of hazard, un-

certainty is connected with possible inaccuracy of data on average annual concentrations of polluting substances in the atmospheric air. At the stage of analysis of relation "dose-response", uncertainties are probably connected with the determination of referential level of influence, transition of results of epidemiological research to the evaluated population of the region, degree of evidence of cancerogenic effect in human and critical organs/systems and hazardous effects.

Conclusion

Consequently, the performed analysis of sanitary-hygienic conditions of living of the Altai Krai population revealed: pollution of the air in the territory of urban and rural populated areas of the region is characterized by the exceeded level of *noncancerogenic risk* conditioned by vanadium pentoxide, carbon black, benzol, formaldehyde - combustion gases of mineral fuel, and also copper, cadmium, benzol, carbon black and formaldehyde oxides contained in the enterprise emissions.

This proves the topicality of the elaboration of hygienic measures not only at the sources of pollution, but also regional planning of populated areas, protection of residential areas from industrial emissions; there is also important the organization in terms of the system of SHM of special measures aimed at targeted preventive medical examinations of child population and standard medical examination of adult population to reveal persons in the groups of exceeded risk of conditioned morbidity.

References

1. Decree of the Government of the Russian Federation of February 2, 2006, No. 60 "On approval of the provision on conducting social and hygienic monitoring". Moscow, February 6, 2006, No. 0235.

2. *Guidance on the assessment of public health risks from exposure to chemicals that pollute the environment (P 2.1.10.1920-04).* Moscow: Federal Center for State Sanitary Epidemiological Supervision of the Ministry of Health of Russia, 2004.

3. Maimulov V.G., Patsyuk N.A., Baskovich G.A. Hygienic assessment of the effect of chemical pollution of the environment of a megacity on the state of children's health. *Hygiene and Sanitation*. 2004; 1: 20-31.

4. Onishchenko G.G. Assessment of the risk of environmental factors affecting health in the system of socio-hygienic monitoring. *Hygiene and Sanitation*. 20024 6(4): 3-5.

5. Rakhmanin Yu.L., Ivanov S.I., Novikov S.M., Revazova Yu. A., Rusakov N.V. Topical problems of the comprehensive hygienic characterization of urban environmental factors and their influence on the population's health. *Hygiene and Sanitation*. 2007; 5(10): 5-6.

6. Rumyantsev G.I., Dimitriyev D.A. Methodological basis for improving the monitoring of the impact of anthropogenic environmental factors on human health. *Hygiene and Sanitation*. 2001; 6(11): 3-5.

7. Tiunov L.A., Kustov V.V. *Toxicology of carbon monoxide.* Moscow, 1980.

Contacts:

Corresponding author: Pashchenko Irina Gennadyevna, Head of the Directorate of Rospotrebnadzor for Altai Krai, Barnaul.
656056, Barnaul, ul. Maksima Gorkogo, 28.
Tel.: +7 (3852) 24-29-96.
E-mail: mail@22.rospotrebnadzor.ru

UDC 616.94:616-006-08-06(571.15)

RISK STRATIFICATION OF INFECTIONS CONNECTED WITH HEALTHCARE DELIVERY IN PATIENTS WITH MALIGNANT NEOPLASMS ON THE BACKGROUND OF CHEMOTHERAPEUTIC AND RADIOTHERAPEUTIC TREATMENT IN ALTAI KRAI

[1]Altai State Medical University, Barnaul

[2]Altai Regional Oncology Center, Barnaul

A.N. Neshatayev [1,2], N.V. Lukyanenko [1], A.F. Lazarev[2], N.Ya. Lukyanenko[1]

The current article presents the results of retrospective analysis of incidence and principles of risk stratification of infections connected with healthcare delivery in patients with malignant neoplasms exposed to chemotherapeutic treatment outpatiently and inpatiently.

Key words: *risk groups, ICHD, chemotherapeutic treatment.*

Infections connected with healthcare delivery (ICHD) are registered in 5-10% of patients of in-patient hospitals and hold the 10th position among causes of mortality. In Russia, according to the official statistics, there areannually registered about 30 thous. cases of ICHD (0,8 per 1000 patients). According to international and Russian specialists in the sphere of ICHD, there truth number constitutes not less than 2-2,5 million people. Some groups of patients are especially vulnerable: newborns, elderly people, patients with severe course of the basic pathology and numerous concomitant diseases, patients exposed to aggressive and invasive medical manipulations, organ transplantations, etc. In these groups, the indexes of ICHD morbidity are much higher.

Patients with malignant neoplasms are one of the ICHD risk groups, taking into account that chemotherapeutic and radiotherapeutic treatment leads to the decrease of immune response to the influence of various bacterial and viral etiological factors. According to V.M. Gelford (2012), basic clinical forms of ICHD in oncological departments are pseudomonas sepsis, acute respiratory distress, ventilation-associated pneumonia. This group of patients (N.I. Petrova, 2009) is also characterized by long-term complications after release from hospital determined by ICHD of viral, parasitic and fungal etiology. According to her data, mycoses generally caused by Candida fungi damage from 10 to 15% patients exposed to chemotherapeutic and radiotherapeutic treatment.

Intensive development of high-tech invasive methods of diagnosis and treatment in combination with wide spread of microorganisms with multiple drug resistance determines the necessity of continuous improvement of systems of surveillance and control.

Patients with ICHD stay in hospital 2-3 times longer than similar patients without infection symptoms. There stay is averagely 10 days longer, the treatment cost is 3-4 times higher, the risk of lethal outcome is 5-7 times higher. The economic damage cause by ICHD is considerable: in the Russian Federation it can lead 10-15 milliard rubles per year (for comparison – annual economic damage from ICHD in Europe constitutes about 7 milliard euro, in the USA – 6,5 milliard dollars).

Epidemiological and economic aspects determine more through study of epidemiological aspects of the course of epidemic processes of infections connected with healthcare delivery in patients with malignant neoplasms on the background of chemotherapeutic and radiotherapeutic treatment aimed at improvement of systems of surveillance and control.

Research objective

Optimization of the information subsystem of epidemiological surveillance of ICHD in patients with malignant neoplasms on the background of chemotherapeutic and radiotherapeutic treatment.

Tasks:

1) To perform retrospective analysis of incidence and reveal ICHD risk groups in patients with malignant neoplasms exposed to chemotherapeutic treatment outpatiently and inpatiently.

2) To perform retrospective analysis of incidence and reveal ICHD risk groups in patients with malignant neoplasms exposed to radiotherapeutic treatment outpatiently and inpatiently.

3) To elaborate criteria of assessment and algorithms of determination of ICHD risk groups patients with malignant neoplasms on the background of chemotherapeutic and radiotherapeutic treatment.

4) Optimization of the information subsystem and managing subsystem in the system of epidemiological ICHD process management in the oncological center.

Materials and methods

Research materials were the data of the official statistical reporting, informational, analytical materials, records and reports andalso results of laboratory microbiology tests selected in 2011-2016 in the Regional State Budgetary Healthcare Institution Altai Regional Oncological Center.

The study included the data of official state statistical reporting and report forms of epy Russian Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing (Rospotrebnadzor) (№2 "Records on infections and infestations", №5 "Records on preventive vaccinations".

The research is based on the retrospective epidemiological analysis of dynamics and structure of morbidity of patients with malignant neoplasms on the background of chemotherapeutic and radiotherapeutic treatment and ICHD in the period from 2011 to 2016.

Causes and conditions of ICHD cases in the Altai Regional Oncological Center in the period from 2011 to 2016 were evaluated by the analysis of records of epidemiological examination of focuses (f. № 391 u) including the data on 5365 patients with malignant neoplasms on the background of chemotherapeutic and radiotherapeutic treatment and 45 ICHD patients in the period from 2011 to 2016.

In 2011-2016, in terms of study, there were selected and examined 120 specimens of clinical material and 360 specimens of external environment of the Altai Regional Oncological Center for microbiological and immunological examinations.

The work implied descriptive evaluative epidemiological, laboratory, statistical methods of research, quantitative method of complex evaluation of ICHD risks and also elements of mathematical modelling.

Results and discussion

The performed retrospective epidemiological analysis of dynamics of morbidity of patients with malignant neoplasms on the background of chemotherapeutic and radiotherapeutic treatment in 2011-2013 compared to 2004 revealed the growth of the overall index of malignant neoplasm incidence by 17,2% (Table 1).

Table 1

Dynamics of morbidity of patients with malignant neoplasms on the background of chemotherapeutic and radiotherapeutic treatment in 2011-2013 compared to 2004

	Malignant neoplasms	2004	2011	2012	2013	Index growth (by 2004), %
1	esophagus	5,5	7,6	6,9	7,9	+43,6
2	gaster	39,6	32,3	37,0	34,9	-11,9
3	middle intestine	17,6	25,6	24,7	24,5	+39,2
4	straight intestine	18,4	19,4	23,6	22,4	+21,7
5	liver	8,3	5,6	5,5	5,9	-28,9
6	pancreatic gland	12,3	14,1	13,8	12,7	+3,3
7	larynx	11,7	13,9	11,6	13,8	+17,9
8	trachea, bronchi, lungs	115,0	107,7	102,9	109,1	-5,1
9	bones, articular cartilages	1,7	0,9	1,8	1,5	-11,5
10	connective and soft tissues	2,1	2,7	2,7	2,9	+38,1
11	skin melanoma	4,1	5,0	5,7	4,4	+7,3
12	other skin neoplasms	43,7	55,6	50,3	56,1	+28,4
13	prostatic gland	26,5	47,9	53,8	62,6	+136,2
14	urinary bladder	17,5	19,7	19,2	20,7	+18,3
15	kidneys	14,9	17,7	20,6	20,4	+36,9
16	thyroid gland	2,9	6,4	7,5	7,0	+141,4
17	lymphoid tumors	11,6	10,5	11,5	9,4	-18,9
18	leukemia	11,2	5,6	10,6	7,5	-33,9
19	Total	413,2	456,8	465,0	484,2	+17,2

The overall index of morbidity constituted 484,2 per 100,0 thous. people in Altai Krai. The maximum increase was registered among the following pathologies: thyroid gland cancer – by 141,4%, prostatic gland cancer – by 136,2%, esophageal cancer – by 43,6%, kidney cancer – by 36,9%. This period was characterized by the reduction of leukemia incidence by 33,9%, lymphoma – by 18,9%, bronchopulmonary system – 5,7%.

The age and sex composition of patients with malignant neoplasms on the background of chemotherapeutic and radiotherapeutic treatment in 2011-2013 is presented in Table 2.

Table 2

Dynamics of age and sex composition of patients with malignant neoplasms on the background of chemotherapeutic and radiotherapeutic treatment in 2011-2013

Age	2011		2012		2013	
	male	female	male	female	male	female
0- 14 years	0,6	0,5	0,5	0,6	0,5	0,4
15-29 years	1,1	1,6	1,1	2,0	0,9	1,7
30-39 years	2,3	4,8	2,0	4,4	2,1	4,7
40-49 years	5,8	9,3	5,7	9,1	4,9	7,9
50-59 years	24,7	23,5	24,3	23,2	23,8	23,4
60-69 years	28,4	21,8	27,8	22,2	34,6	25,3
70 years and older	37,1	38,5	38,6	38,4	33,1	36,7
Total	49,2%	50,8%	46,9%	53,1%	48,8%	51,2%

The structure of age and sex composition of patients with malignant neoplasms on the background of chemotherapeutic and radiotherapeutic treatment in 2011-2013 was not exposed to changes.

The specific gravity of male patients constituted from 46,9% to 49,2%, female patients – from 50,0% to 53,8%.

The overall structure of patients was prevailed by elder age groups over 70 - 36,7%, 60-69 - 25,3%, children under 14 – 0,4%.

The most spread oncological pathologies among men were prostatic gland cancer – 12,9%, urinary bladder cancer – 4,3%, kidney cancer – 4,3%, lung cancer – 22,5%, skin neoplasms – 11,6%. Among women there prevailed mammary gland cancer – 17,0%, uterine body and uterine cervix cancer – 13,2%, thyroid gland cancer – 8,0%.

Prevailing methods of treatment of patients with malignant neoplasms were the methods of combined complex treatment using surgical method – 76,9% (Table 3).

The treatment did not require surgical intervention in 23,1% of patients including only radial method – 18,1%, chemotherapeutic and radial methods – 20,3%.

According to the obtained data and in order to perform retrospective study of hospital-acquired infection incidence and routes and factors of infection, our objective was to determine ICHD risk groups.

According to the data of world and Russian statistics, the most significant ICHD risk groups were patients undergoing combined complex treatment implying surgical method – 76,9% (first group – 4289 patients). The given group of patients was exposed to lung ventilation with risk of ventilation associated pneumonia. Due to numerous intravenous injections, these patients had the risk of catheter-associated purulent-septic pathologies. According to literature, ventilation associated pneumonia in this group is registered in 20-50% of patients.

The second risk group was the group of patients with prostatic gland and urinary bladder cancer – 145 patients exposed to the urinary bladder catheterization. According to the data of world and Russian statistics, the risk of purulent-septic complications in this group by the urinary bladder catheterization and suprapubic urinary catheter constitutes from 1,5% to 38,3%.

The third risk group were patients with straight intestine, rectosigmoid junction and anus cancer – 185 patients. 25,0% of patients in the given group had statistically significant risk of purulent-septic infections including endogenous genesis.

The fourth risk group consisted of patients with lymphatic and blood-forming tissues cancer – 176 patients, which has the risk of purulent-septic infections – 70,6%. 81,8% exposed to chemotherapeutic treatment only.

Conclusion

Thus, there has been determined four ICHD risk groups:

1. Patients exposed to combined complex treatment implying surgical method – 4289 patients.
2. Patients with prostatic gland and urinary bladder cancer exposed to the urinary bladder catheterization – 145 patients.
3. Patients with straight intestine, rectosigmoid junction and anus cancer – 185 patients.
4. Patients with lymphatic and blood-forming tissues cancer – 176 people.

References

1. Karpenko A.G., Novikova O.G., Gerasimova M.S. Microbiological support of epidemiological surveillance in COD MH RT. *Oncology Bulletin of the Volga Region*. 2012; 1.

Table 3

Methods of treatment of patients with malignant neoplasm in 2013

Localization, nosological form	abs. number	Implying surgical method, %			Without surgical treatment %			
		only surgical treatment	combined/ complex treatment	total with surgical treatment	only radial method	chemo-radial method	total with radial method	only drug method
All malignant tumors	5578	44,9	32,0	76,9	18,1	2,2	20,3	2,8
including children (under 14)	21	19,0	23,8	42,9	0,0	9,5	9,5	47,6
Oral and pharyngeal cavity	57	10,5	29,8	40,4	29,8	29,8	59,6	0,0
Esophagus	18	55,6	22,2	77,8	0,0	22,2	22,2	0,0
Gaster	208	38,9	61,1	100,0	0,0	0,0	0,0	0,0
Straight intestine, rectosigmoid junction, anus	185	53,0	38,4	91,4	3,2	5,4	8,6	0,0
Larynx	72	13,9	45,8	59,7	34,7	5,6	40,3	0,0
Trachea, bronchi, lungs	355	46,5	47,3	93,8	1,7	4,5	6,2	0,0
Skin melanoma	118	69,5	30,5	100,0	0,0	0,0	0,0	0,0
mammary gland	673	21,4	78,6	100,0	0,0	0,0	0,0	0,0
Uterine cervix	239	22,2	13,8	36,0	54,0	10,0	64,0	0,0
Uterine body	309	36,9	56,6	93,5	5,8	0,6	6,5	0,0
Ovaries	80	13,8	86,3	100,0	0,0	0,0	0,0	0,0
Urinary bladder	145	32,4	67,6	100,0	0,0	0,0	0,0	0,0
Lymphatic and blood-forming tissues	176	0,0	4,0	4,0	0,0	14,2	14,2	81,8

2. Gelford V.D. Infectious complications in cancer patients. *Practical oncology* 2009; 3 (10).

3. Tutelyan A.V., Pisarev V.M., Minayeva N.Z., Gaponov A.M., Gracheva A.N., Solonova G.G. Generalized antibiotic tolerance in hematological and oncological diseases accompanied by immunocompromentation: a new problem in connection with the provision of medical care. *Annals of the Russian academy of medical sciences.* 3 (71).

4. Akimkin V.G., Tutelyan A.V., Brusina Ye.B. Actual directions of scientific research in the field of non-specific prevention of HAI. *Epidemiology and infectious diseases*. 2014; 2.

5. Perevodchikova N.I., Gorbunova V.A., ed. *Guidance to chemotherapy for tumor diseases*.

Contacts:
Corresponding author: Lukyanenko Natalya Valentinovna, Doctor of Medical Sciences, Professor of the Department of epidemiology, microbiology and virology of ASMU, Barnaul.
656056, Barnaul, ul. Papanintsev, 126.
Tel.: (385) 256-69-24.
E-mail: natvalluk@mail.ru

UDC 613.22:613.955(470-20)+(571.15)

ASSESSMENT OF EATING BEHAVIOR IN PUPILS OF MOSCOW AND ALTAI KRAI AND THE ROLE OF FAMILY AND SCHOOL IN THE FOOD CULTURE FORMATION

[1]National Medical Research Center of Children's Health, Moscow
[2]Altai State Medical University, Barnaul

Zh.Yu. Gorelova[1], S.P. Filippova[2], Yu.V. Solovyeva[1], A.N. Plats-Koldobenko[1], T.A. Letuchaya[1], I.P. Saldan[2], O.V. Zhukova[2]

The article presents the data on the assessment of nutrition of children and teenagers in modern conditions. It has been revealed, that the products, meals and ready-to-serve foods provided at school do not satisfy taste preferences of pupils, there lacks the tradition of home and school meal. Thus, it is necessary not only to inculcate eating habits, but also to correct the formed ones – both at school and at home. A considerable role in this process is given to the family.
Key words: *eating habits, ration, tradition.*

Researches on healthy nutrition of children and teenagers in modern conditions show, that the school menu not always satisfy taste preferences of pupils, there lacks the tradition of home and school meal. Thus, it is necessary not only to inculcate eating habits, but also to correct the formed ones – both at school and at home. A considerable role in this process is given to the family [3, 10].

Research objective

Considering the problem topicaly, there were conducted researches of the

Federal State Autonomous Institution "National Medical Research Center of Children's Health" of the Ministry of Health of the Russian Federation, I.M. Sechenov First Moscow State Medical University, FSBEI HE ASMU of the Ministry of Health of the Russian Federation, aimed at the generalization and analysis of the information on taste preferences of 1-11 form pupils in **220 schools** in different Moscow districts (Central Administrative District, Northern Administrative District, North-East Administrative District, Eastern Administrative District, South-East Administrative District, Western Administrative District, North-West Administrative District, Troitsky and Novomoskovsky Administrative Districts, Zelenogradsky Administrative District) and municipal general educational institutions of Altai Krai.

Materials and methods

In Moscow schools there was evaluated the consumption of every current menu item for the moment of study, including products from the school shop and outside the school (favorite courses eaten at home). There were used specifically developed questionnaires. According to the results of the current study there was created the consolidated report (percentage of food consumption) on the basis of questionnaire data obtained during the monitoring of food consumption by pupils of various age groups in canteens of educational institutions. In terms of the data comparison, there were also used the results obtained by the determination of the rate of food waste by means of weighing [3].

The study included the data on 202 385 course helpings, 129 159 out of them – breakfast helpings and 73 226 lunch helpings, given according to the current menu depending on the age of pupils [3, 6, 7, 8].

The number of pupils involved into the study constituted 49 952, the number of questionnaires of pupils and parents – 751, including reports on the quality of food, assessment of subsidized meals, questionnaires on the evaluation of the coffee shop range – 172, observations of teachers, service staff, number of acts on the study management – 567/total – 1318/, the results of monitoring of school nutrition were used for the comparison analysis [1, 3, 4, 9, 10], and the results presented by the Razymovsky MSUTM – Ivanova V.N., Mogilny M.P., 2015 and the computer agency KAPITAN, 2012, (http://www.1cp.ru/m/sm/index.php).

In terms of the analysis of the obtained data, there was prepared the "Analytical report on the rationale for the target audience selection and the results of the analysis of eating behavior in pupils of Moscow institutions of general education" for the Moscow Department of Education (See the official website of the Moscow Department of Education).

Results and discussion

According to the monitoring results, taste preferences of pupils can be conventionally divided into three groups:

- dishes, which children practically do not eat; the rate of consumption of these dishes constitutes less than 35% of the total amount of food served;

- dishes, which children eat with pleasure, but in the amount lesser than the amount prescribed in the current menu, the rate of consumption of these dishes constitutes 50-65%;
- dishes, which children eat with great pleasure, the rate of consumption of these dishes constitutes 70% and more.

The number of dishes (totally, there were examined 159 ready-to-serve foods, dishes, food products) according to the current menu considering the rate of consumption was divided into the following proportions: 22% - dishes, which children practically do not eat, 23% - dishes, which children eat with pleasure, 55% - dishes, which children eat with great pleasure. The obtained data prove the necessity and reasonability of the elaboration of new food rations considering modern taste preferences of pupils, including favorite dishes and excluding less consumed dishes at school, considering home nutrition and following daily physiological needs of pupils and principles of succession of home and school nutrition, the so called "school-family" menu [2,3,5].

The elaboration of the new daily ration provides for the reasonability of exclusion of dishes, which consumption rate constitutes less than 50% ("squash spread", "spinach soup", "poached fish", "starch drink", etc.), considering the issue on the reduction of exclusion of dishes important for the adherence to the nutrition value, the consumption of which ranges within the interval of 50-65%, with the compensation of the number of nutrients by the addition of other products to the menu. For example, by the reduction of "milk millet porridge" from 150g to 80g the menu should include a cottage cheese pancake for breakfast. Dishes with the rate of consumption over 70% should be left in the new menu without alterations, as they are the most popular according to taste preferences.

Such dishes include: "macaroni baked with cheese", "vegetable salad with beetroot", "salad with fresh tomatoes and cucumbers with vegoil", "borsch with cabbage and potatoes", "Siberian borsch with beef", "beef stroganoff with macaroni", "Leningradsky pickle soup", "potato soup with meatballs", "chicken soup", "chopped chicken meatballs", "goulash", "chopped meat patties (beef)", "chopped chicken patties", "meat pilaf", "mashed potatoes", "boiled rice", porridge "Druzhba", "vegetable ragout", "dried apricots compote", "cacao drink with milk", "school pizza", "chocolate cupcake", "cottage cheese pancakes", "cherry pie" and others (87 out of 159 dishes and ready-to-serve foods – 55%).

According to the results of the inquiry, the prevailing products in the day ration bought additionally at retail outside the school and home during the day are chips, carbonate water, bread rolls, sweets. In the school shop – confectionery (20%), bakery (25%), drinks (19%), sandwiches (36%).

In Altai Krai, there was studied the actual nutrition of pupils at the age 12-17 years (n=257) considering additional nutrition at home by means of the analysis of frequency of food consumption, analysis of menus of the organized group [9]. The main group included 125 pupils of schools with modernized technological equipment of the nutrition unit. The group of comparison included 132 pupils of schools without modernized nutrition unit. The parents of pupils (n=257) also took part in the study and answered to the questionnaire: "Information on nutrition and eating behavior". The study revealed, that the modernization of school canteens allowed to improve the structure of nutrition, increase the consumption of a number of nutrients, the quality of nutrition in the main group was subjectively evaluated higher than in the group of comparison ($p<0,001$).

However, in spite of the conducted measures, the nutrition of 79,8±2,5% of pupils is not balanced by the content of nutrients and energy, which is determined by taste preferences of teenagers and nutrition outside the school [1, 4, 9]. Thus, in the main group, the indexes of the energy share of proteins and fats turned out to be higher, as well as the consumption of meat and meat products, fish, sea products, confectionaries, plant products. At the same time, the compared groups were characterized by low level (lower than the norms of physiological needs) of consumption of meat, milk and dairy products, fish and fish products, eggs, which is also determined by the refusal of pupils in the school canteen of such products as steam-cooked "beef patties", steam-cooked "beef meat balls", steam-cooked "fish patties".

It has been long accepted, that steam-cooked dished cooked in the convection steamer retain nutrients, and as everything is cooked without fat, there is totally realized the concept of healthy food. The choice is, unfortunately, made for such meat products as sausages. In the group of comparison, there was registered insufficient consumption of proteins (7,5%; $p=0,0198$). The consumption of proteins by young boys constituted 109%, by girls – 93,5% of the norms of physiological needs ($p=0,014$). In boys 12,5% of calories constitute animal proteins, in girls – 11,5%. In 7,0±1,6% of pupils insufficient consumption of essential amino acids, low content of methionine in 58,8±3,1%. In the main group there are observed higher rates of fat consumption (136% of the norms of physiological needs) than in the comparison group – 112,5% ($p=0,0216$) and sodium ($p=0,0242$). Herewith, the contribution of school nutrition to the overall fat consumption rate constituted 22,1% and did not differ significantly in both groups ($p>0,05$).

The consumption of PUFAs constituted 7,3±0,22% of the daily ration calories (by the norm 5-14%), while the correlation of PUFA omega-6/ omega-3 constituted 16:1 due to the shortage

of omega-3 PUFA. In 36,2±3,0% of pupils there was registered the redundancy of the energetic content of the ration. With the age, the fat component in the ration increased: in pupils at the age of 12-14 years, proteins provide 11,8% of calories, fats – 36%, carbohydrates – 52,1%, at the age of 15-17 years: proteins – 12,6%, fats – 38,1%, carbohydrates – 49,3% (optimal rate is 10-15%, 30-32% 55-60% respectively). This is on the background of lower consumption of fruit by teenagers of 15-17 years in comparison with teenagers of 12-14 years, 255 g/day and 322 g/day respectively (p=0,014) and higher consumption of oils and fats by elder teenagers 26g/day in comparison with younger teenagers 18g/day (p=0,036) [9].

The insufficiency of consumption of carbohydrates in the main group and the group of comparison constituted 86,4% and 90,7% of the norm of physiological needs, while in teenagers at the age of 12-14 years, the rate of consumption corresponds to the norm of physiological needs, and in teenagers at the age of 15-17 years, it constitutes 77,5% of the norm (p=0,001). The consumption of dietary fiber: 95% of the norm in boys, 81% of the norm in girls.

Pupils do not follow the recommended frequency of nutrition: 36,2% eat 2-3 times a day; 40,4% - 4-6 times; 23,2% do not follow constant frequency and eat from 2 to 6 times a day.

The contribution of school nutrition to the structure of food consumption constitutes 20,8%, which determines the necessity of more active participation of parents (family) in the formation of eating habits and ration of pupils. Moreover, 73% of pupils at the age of 12-17 years, who had taken part in the research, mentioned, that the parents' lifestyle is an example for them. The study of eating behavior of parents revealed: the specific gravity of those, who placed the quality of products on the first position among the characteristics, which they consider by the choice of products, constituted 35%, the second position was occupied by family budget – 19,2%, the third and the fourth positions – taste habits and personal wishes – 14,8%.

The nutrition value, knowledge of the product's usefulness and doctor's advice were rarely placed on the first position. The consumption of fresh fruit and vegetables constituted 114,3 g/day and 128,6 g/day per person respectively, which is lower than the norms recommended by the WHO. Insufficient consumption of milk and curdled dairy products - in 52,5±3,1% of parents, normal – in 17,9±2,4%. During the recent year, 39,9% of parents have been trying to use less salt, among urban population - 45,3±4,4%, among rural population - 33,3±4,2% (2I=6,1; p<0,05). 29,2±2,8% constantly use iodine-treated salt, 46,7±3,1% use it sometimes and 24,1±2,7% - very rarely. 13,6% of parents do not follow the recommended frequency of meals and eat 1-2 times a day. Irregular food consumption is registered in 62,3% of respondents, 26,5% have restricted time, 21,4 often overeat. Only 19,8% of parents regularly follow the regime of nutrition and receive vitamin-mineral complexes [9].

Conclusion

Consequently, the assessment of nutrition of children and teenagers in modern conditions and their eating behavior shows, that the number of products, dishes and ready-to-serve foods provided at school do not satisfy taste preferences of pupils (are not liked, are not eaten or eaten incompletely), there lacks the tradition of home and school meal. It determines the necessity of teaching parents, children and teachers to correctly form modern principles and habits of healthy nutrition, to consider eating preferences of pupils and to contribute to teaching the use of "school-family menu".

The example of adults is extremely important in the formation of principles and skills of healthy nutrition, healthy and regular habits. Family breakfasts, lunches and dinners are more pleasant for a child than eating alone. Parents are the main ideal of conduct for a child.

References

1. Analytical report on the state of the school nutritionsystem in the Russian Federation and the progress of implementing the Experiment in all subjects of the Russian Federation (based on the data of the All-Russian monitoring of the organization of school nutrition in 2011).

2. Kuchma V., Gorelova Z. International experience of organizing school nutrition. *Current pediatrics*. 2008; 7(2):14-22.

3. Kuchma V. R., Gorelova Zh. Yu., Uglov S.Yu., Anufrieva T.A. Sacientific substantiation and development of a two-week daily diet of Moscow students. Issues of school and university medicine and health. 2015; 4: 46-47.

4. Scientific library CyberLeninka: http://cyberleninka.ru/article/n/konstruirovanie-i-otsenka-potrebitelskih-svoystv-funktsionalnyh-pischevyh-produktov-dlya-shkolnogo-pitaniya#ixzz3c2INQWZM.

5. Zigmund Ye. Which is good for a Swede. Available at: http://www.itogi.ru/obsch/2011/45/171427.html.

6. Mogilny M.P., Tutelyan V.A., ed. *Collection of technical specifications. Collection of recipes for products for students in all educational institutions.* Moscow: DeLi print; 2011.

7. Mogilny M.P., Tutelyan V.A., ed. *Collection of technical specifications. Collection of recipes for dishes and culinary products for children in pre-school organizations.* Moscow: DeLi print; 2012.

8. Titov Ye.I., Mitaseva L.F., Pyltsova L.A. Technological instruction for the production of culinary products for nutrition of children and ad-

olescents of school age in organized collectives. Moscow; 2006.

9. Filippova S.P. *Hygienic assessment of pupils nutrition and the effectiveness of the regional program of school feeding modernization in modern conditions (on the example of the Altai Krai).* [dissertation]. Omsk, 2015.

10. Kuchma V., Gorelova J. «International experience of organizing school nutrition». *The Union of Pediatricians of Russia Scientific Practical Journal*. 2009. 60-66.

Contacts

Corresponding author: Filippova Sofya Petrovna, Candidate of Medical Sciences, Associate Professor of the Department of hygiene, ecology and life safety fundamentals of ASMU, Barnaul.
656056, Barnaul, per. Nekrasova, 65.
Tel.: (385) 224-97-19.
E-mail: sofya.filippova@mail.ru

UDC 613.31:543.3:351.77 (571.15)

THE ASSESSMENT OF RISK OF DRINKABLE WATER CHEMICAL POLLUTION TO THE HEALTH OF ALTAI KRAI POPULATION

Center of hygiene and epidemiology in Altai Krai, Barnaul

T.I. Gubareva, A. Yu. La, Yu.A. Kapayeva, L.A. Skripkina, N.Ye. Soldatova

There was performed the hygienic assessment of the level of drinkable water chemical pollution on the territory of Altai Krai and revealed the most troubled territories consuming chemically polluted drinkable water. The data obtained by means of social-hygienic monitoring allowed to adjust the plan of monitoring laboratory tests of drinkable water of centralized water supply systems in populated areas.
Key words: *social-hygienic monitoring, drinkable water, chemical substances, troubled territories.*

Water is an essential element of the environment having a significant impact on human health and activity, it is the basis for origin and maintenance of all life. Providing the population with drinkable water of satisfactory quality is one of the main social-hygienic issues. Drinkable water should be safe in epidemiological and radiation respect, harmless in chemical composition and should have favourable organoleptic properties. It is believed that up to 80 per cent of all chemical compounds entering the outside environment sooner or later get into natural water with industrial, household and storm drains. Numerous studies have established that drinkable water anthropogenic pollution along with other environmental factors is an intensive factor of impact on human health.

Objective

To conduct a hygienic assessment of the level of drinkable water chemical pollution on the territory of Altai Krai to reveal the most troubled territories and amount of population consuming chemically polluted drinkable water.

Materials and methods

In 2016, 34 902 studies with a multiplicity of 12 samples per year were conducted at 372 monitoring points on the distribution network of centralized utility and drinking water supply systems within the framework of social-hygienic monitoring system in Altai Krai. To assess the direct influence of the quality of drinkable water on population health, on administrative territories of Altai Krai, a calculation and population health risk assessment (the assessment of the probability of developing a threat to a person's or future generations' life or health) was carried out, this risk being preconditioned by drinkable water chemical pollution. In this study, the assessment of risk was based on average annual chemical substances concentrations on the distribution network. The studies of drinkable water on distribution network for the presence of lead and cadmium were conducted at 11 districts of Altai Krai at 18 populated areas, the amount of population under studies equaled to 440 019 persons. The studies for the presence of lead, cadmium and arsenic were conducted at 4 districts of Altai Krai at 4 populated areas, the amount of population under studies equaled to 189 810 persons, making 9.0 per cent of the total amount of population using centralized utility and drinking water supply. The studies for the presence of lead, cadmium, arsenic and chromium (6+) were conducted at the town of Aleysk, the amount of population under study equaled to 28 372 persons. All studies were performed by accredited laboratories with the use of standardized analytical methods. Assessment of risk was carried out in accordance with the provisions of P 2.1.10.1920-4 "Human Health Risk Assessment from Environmental Chemicals".

Results and discussion

It was established that four groups were registered in the analysis of sanitary-chemical parameters of controlled substances.

The *first group* is for substances of hazard classes 1 and 2 (arsenic, lead, boron, nickel, nitrites) with concentration exceeding 1 TLV. This group includes 7 territories of Altai Krai: Pervomaysky, Khabarsky, Rebrikhinsky, Pavlovsky, Shipunovsky, Kamensky Districts, the city of Barnaul.

The *second group* is for substances of hazard class 3 (iron, manganese, magnesium, nitrates, chlorine) with concentration exceeding from 1 to 2 TLV. This group includes 19 territories: Ust-Kalmansky, Troitsky, Topchikhinsky, Talmensky, Tabunsky, Rebrikhinsky, Pankrushikhinsky, Kulundinsky, Krasnoshchyokovsky, Kalmansky, Kuryinsky, Bystroistoksky, Altaysky, Aleysky, Loktevsky, Zavyalovsky Districts, the city of Rubtsovsk, the towns of Slavgorod, Aleysk.

The *third group* is for substances of hazard class 3 (iron, manganese, nitrates) with concentration exceeding from 2 to 5 TLV. This group includes 10 territories: Romanovsky, Novichikhinsky, Nemetsky National, Yeltsovsky, Burlinsky, Kosikhinsky, Shelabolikhinsky Districts, the towns of Novoaltaysk, Kamen-na-Obi, Belokurikha.

The *fourth group* is for substances of hazard class 3 (nitrates, iron, manganese) with concentration exceeding 5 TLV. This group includes 26 territories: Shipunovsky, Khabarsky, Tyumentsevsky, Suyetsky, Soltonsky, Soloneshensky, Sovetsky, Smolensky, Rubtsovsky, Pospelikhinsky, Pavlovsky, Mamontovsky, Krutikhinsky, Krasnogorsky, Kamensky, Zonalny, Zarinsky, Tselinny, Zalesovsky, Yegoryevsky, Blagoveshchensky, Biysky, Pervomaysky Districts, the town of Zarinsk, the cities of Biysk and Barnaul.

No exceedance of concentration of above mentioned substances was found in populated areas of Bayevsky, Volchikhinsky, Zmeinogorsky, Klyuchevsky, Kytmanovsky, Mikhaylovsky, Petropavlovsky, Rodinsky, Slavgorodsky, Togulsky, Tretyakovsky, Uglovsky, Ust-Pristansky, Charyshsky Districts and the town of Zmeinogorsk.

The total individual carcinogenic risk due to peroral route of lead, cadmium, arsenic with drinkable water is within permitted limits.

The individual carcinogenic risk at the town of Novoaltaysk, and Novichikhinsky, Talmensky, Troitsky, Shipunovsky Districts is up to the first range (the individual risk throughout life is equal to or less than 1x10-6). The individual carcinogenic risk at the town of Zmeinogorsk, and Petropavlovsky, Kamensky, Krutikhinsky, Kuryinsky, Pervomaysky, Tretyakovsky, Krasnoshchyokovsky, Pospelikhinsky, Loktevsky Districts is up to the second range (the individual risk throughout life is more than $1x10^{-6}$, but less than $1x10^{-4}$).

Non-carcinogenic risks were estimated in 78.1 per cent of Altai Krai population using centralized drinking water supply. The number of people who underwent the assessment of risk of non-carcinogenic effects was 1,653,826. As a consequence of drinkable water use in Altai Krai, the level of non-carcinogenic risk from chemical substances in adult population exceeds the permitted value (HQ>1) in Rubtsovsky, Nemetsky National Districts. The contribution to non-carcinogenic risk in adult population is due to the receipt of nitrates from 71.4 per cent in Nemetsky National District to 91 per cent in Rubtsovsky District.

The level of non-carcinogenic risk from substances in child population (from 6 to 18 years) exceeds the permitted value (HQ>1) in Rubtsovsky, Nemetsky National, Loktevsky Districts. The contribution to non-carcinogenic risk in is due to the receipt of nitrates from 70 per cent in Nemetsky National District to 89.2 per cent in Rubtsovsky District. The level of non-carcinogenic risk in child population (from 0 to 6 years) exceeds the permitted value (HQ>1) in Kamensky, Tyumentsevsky, Shelabolikhinsky, Altaysky, Yegoryevsky, Zmeinogorsky, Krasnoshchyokovsky, Loktevsky, Mamontovsky, Nemetsky National, Rubtsovsky, Tretyakovsky Districts. The main contribution to non-carcinogenic risk in child population is due to the receipt of nitrates and fluorine.

In the case of simultaneous receipt of chemical substances with drinkable water the following critical organs are identified among adults and children: blood and blood-forming organs, cardiovascular system, skeletal system and teeth, immune system, central nervous system, hormone system, reproductive system, morphosis, liver, kidneys, skin and mucous membranes, gastrointestinal tract. Total risk indices in case of simultaneous chemical substances receipt in their impact on critical organs and systems in adult population exceed permissible values in the influence on blood and cardiovascular system in Rubtsovsky, Nemetsky National Districts. In child population (from 6 to 18 years), total risk indices exceed the permissive value (>1) for blood and blood-forming organs, cardiovascular system in Rubtsovsky, Nemetsky National and Loktevsky Districts. For child population (from 0 to 6 years), total risk indices exceed the permissive value (>1) for blood and blood-forming organs, cardiovascular system in Altaysky, Yegoryevsky, Zmeinogorsky, Krasnoshchyokovsky, Loktevsky, Mamontovsky, Nemetsky National, Rubtsovsky, Tretyakovsky Districts; for skeletal system in Kamensky, Pavlovsky, Slavgorodsky, Tyumentsevsky, Khabarsky, Shelabolikhinsky Districts.

Conclusions

The results obtained make it possible to conclude that:

1. In Altai Krai the levels of *total carcinogenic risk* from lead, cadmium, arsenic, chromium (6+) are within permitted limits.

The *individual carcinogenic risk* at the town of Novoaltaysk, and Novichikhinsky, Talmensky, Troitsky, Shipunovsky Districts is up to the first range of risk (the individual risk throughout life is equal to or less than $1x10^{-6}$), these risk levels does not require additional measures, but are subject to periodic monitoring.

The *individual carcinogenic risk* at the town of Zmeinogorsk, and Petropavlovsky, Kamensky, Krutikhinsky, Kuryinsky, Pervomaysky, Tretyakovsky, Krasnoshchyokovsky, Pospelikhinsky, Loktevsky Districts is up to the second range of risk (the individual risk throughout life is more than $1x10^{-6}$, but less than $1x10^{-4}$). These levels are subject to constant monitoring.

2. The levels of *non-carcinogenic risk* in adult population exceed the permitted value (HQ>1) for nitrates in Rubtsovsky, Nemetsky National Districts. Children are less resistant to influence of chemical pollutions of environmental objects, which include drinkable water. The level of non-carcinogenic risk in child population (from 6 to 18 years) from chemical substances exceeds the permitted value (HQ>1) for *nitrates* in Rubtsovsky, Nemetsky National,

Loktevsky Districts. In child population (from 0 to 6 years), the level of non-carcinogenic risks from substances exceeds the permitted value (HQ>1) for nitrates and fluorine in Kamensky, Tyumentsevsky, Shelabolikhinsky, Altaysky, Yegoryevsky, Zmeinogorsky, Krasnoshchyokovsky, Loktevsky, Mamontovsky, Nemetsky National, Rubtsovsky, Tretyakovsky Districts and are estimated as elevated.

3. Total risk indices in case of simultaneous chemical substances receipt in their impact on critical organs and systems in adult population exceed permissible values in the influence on blood and cardiovascular system in Rubtsovsky, Nemetsky National Districts.

4. In child population (from 6 to 18 years), total risk indices exceed the permissive value (>1) for blood and blood-forming organs, cardiovascular system in Rubtsovsky, Nemetsky National, Loktevsky Districts. For child population (from 0 to 6 years), total risk indices exceed the permissive value (>1) for blood and blood-forming organs, cardiovascular system in Altaysky, Yegoryevsky, Zmeinogorsky, Krasnoshchyokovsky, Loktevsky, Mamontovsky, Nemetsky National, Rubtsovsky, Tretyakovsky Districts; for skeletal system in Kamensky, Pavlovsky, Slavgorodsky, Tyumentsevsky, Khabarsky, Shelabolikhinsky Districts.

5. This information material is appropriate for use when planning laboratory studies of drinkable water in a distribution network in population centres and making management decisions through the Federal Law No 294-FZ of December 26, 2008 (as amended by the Federal Law No 277-FZ of July 3, 2016).

Thus, the data of social-hygienic monitoring made it possible to numerically assess the negative impact on health due to drinkable water from centralized systems in Altai Krai, to identify troubled territories, and also to adjust the plan of monitoring laboratory tests of drinkable water of centralized water supply systems in population centres.

Contacts
Corresponding author: Gubareva Tatyana Ivanovna, Chief Physician of the Center of hygiene and epidemiology in Altai Krai, Barnaul.
656043, Barnaul, per. Radishcheva. 50.
Tel.: (3852) 353993.
E-mail: mail@altcqe.ru

UDC 616.831.9-002:616.157-036.22 (571.15)

CURRENT EPIDEMIOLOGICAL SITUATION ON BACTERIAL MENINGITIS IN ALTAI KRAI

[1] Altai State Medical University, Barnaul
[2]Regional reference center of bacterial meningitis monitoring

Yu.A. Kozyanova[1], T.V. Safyanova[1], S.V. Shabanova[2]

There was performed the assessment of current situation on bacterial meningitis morbidity in Altai Krai for the period of 2011-2016 and revealed some peculiarities of the epidemic process in the territory of the region.
Key words: *bacterial meningitis, retrospective analysis, pneumococcal disease, morbidity.*

Streptococcus pneumoniae is a widespread etiologic agent causing a large range of human infectious diseases, which is connected with an abundance of serotypes preconditioning the duration of carriage and development of severe forms of diseases [1; 4]. Meningitis, as one of the clinical forms of pneumococcal disease, is the issue of concern of the whole world, as it causes over 60 thousand deaths per year and a high level of disability among children under 5 years old [3]. The ratio of etiologic pathogens to meningitis is not always the same and depends on climatic conditions, the level of immunoprophylaxis, territory, specifically the tension of epidemiological situation. In Altai Krai, the spread of pneumococcal disease has not been studied as well as in the Russian Federation on the whole, and it has determined the objective of this work.

Research objective is the assessment of current epidemiological situation on bacterial meningitis (hereinafter BM) morbidity in Altai Krai population in 2011-2016.

Materials and methods

Retrospective epidemiological analysis of BM morbidity is based on data of official statistics of Ministry of Health in Altai Krai using the report form No 12 "Records on the number of diseases registered among patients living in the area of medical organization service" and data of Regional reference center of bacterial meningitis monitoring.

The materials of research were subjected to statistical processing. The calculation of extensive and intensive morbidity rates, coverage errors (m), average data (X), Student's t-test has been made. In all statistical analysis procedures, the critical significance level was taken as 0.05.

Results and discussion

For the period from 2011 to 2016, a tendency to bacterial meningitis morbidity reduction is observed in Altai Krai population (figure 1). Thus, in 2016 morbidity rate was 1.4±0.25 per 100 thousand of population, in 2011 the rate was 3.8±0.40 per 100 thousand of population. The peak of morbidity was found in 2011 and amounted to 3.8±0.40 per 100 thousand of population. The average long-term bacterial meningitis morbidity rate for the period under study was 2.1±0.12 per 100 thousand of population. For the period of 2011-2016, the rate of decrease was 63.2 per cent, the average annual rate of decrease was 10.5 per cent. However, for the period from 2015 to 2016, the morbidity rate increased by 27.3 per cent from 1.1±0.22 per 100 thousand of population in 2015 to 1.4±0.25 per 100 thousand of population in 2016.

When assessing BM morbidity rate in various age groups, it was found that in the period under study, the highest average morbidity rate was registered among children (from 0 to 14 years) and amounted to 2.9±0.35 per 100 thousand of the corresponding population. The average morbidity rate among adolescents (from 15 to 17 years) was equal to 0.8±0.43 per 100 thousand of the corresponding population. From 2012 to 2016, no cases of BM morbidity among adolescents were registered. The morbidity rate among adults (18 years and more) averaged 1.5±0.11 per 100 thousand of the corresponding population (figure 2).

For the period of 2011-2016, the majority of people having bacterial meningitis was among adult population 76.2±2.45 per cent (figure 3). Among adolescents, this percentage was 1.0±0.57 per cent. The average percentage of children having bacterial meningitis was 22.8±2.42 per cent.

In 2014, in Altai Krai Regional reference center of bacterial meningitis monitoring was created, where bioassays from patients with bacterial meningitis were sent from around the region. According to the center's report, the average percentage of etiologic agent detection in the obtained samples was 54.7±6.84 per cent (Table 1). In the isolated cultures, the share of meningococcal meningitis was 44.8±9.23 per cent, the share of pneumococcal meningitis was 55.2±9.23 per cent.

Conclusion

By the results of the assessment of current epidemiological situation on BM morbidity, it can be concluded that in Altai Krai for the period from 2011 to 2016 bacterial meningitis morbidity had a general tendency to decrease. However, for the

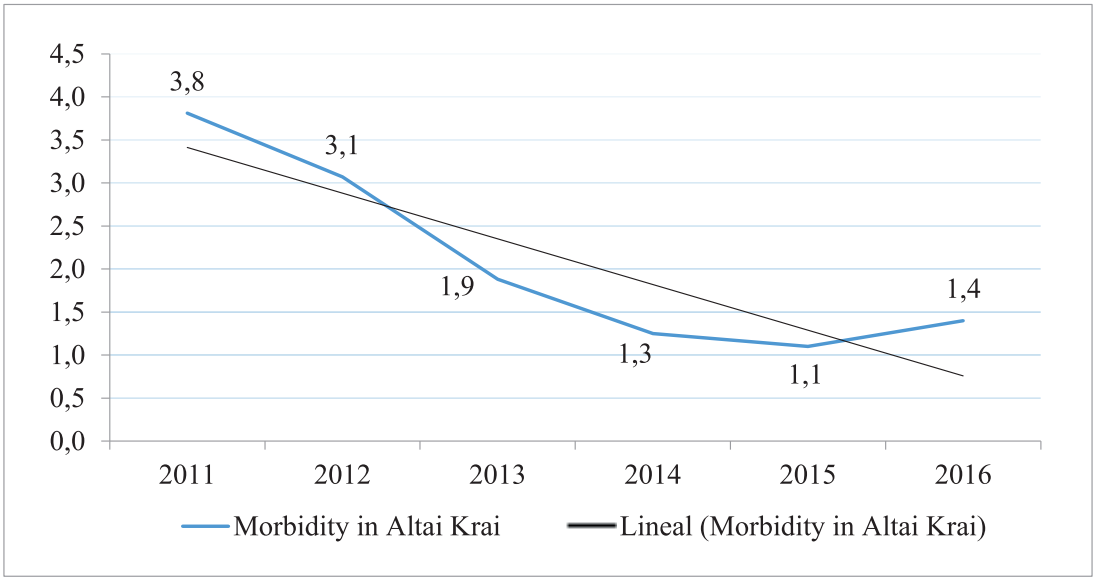

Figure 1. The dynamics of bacterial meningitis morbidity in Altai Krai for the period of 2011-2016 (per 100 thousand of population)

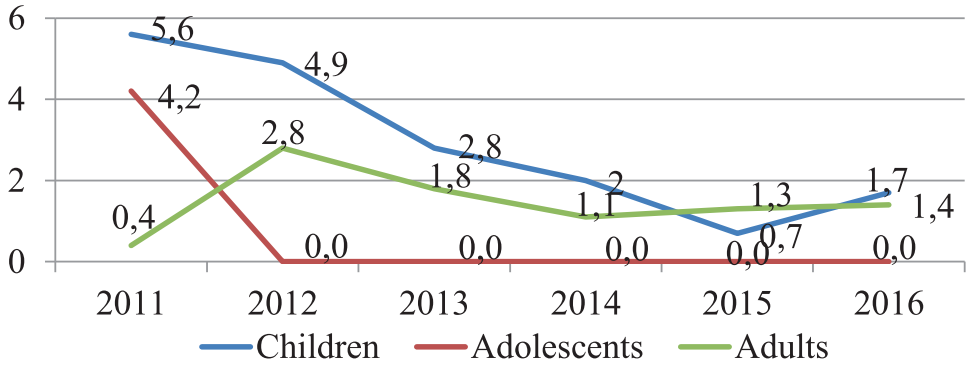

Figure 2. The dynamics of bacterial meningitis morbidity among various age groups in Altai Krai for the period of 2011-2016. (per 100 thousand of the corresponding population)

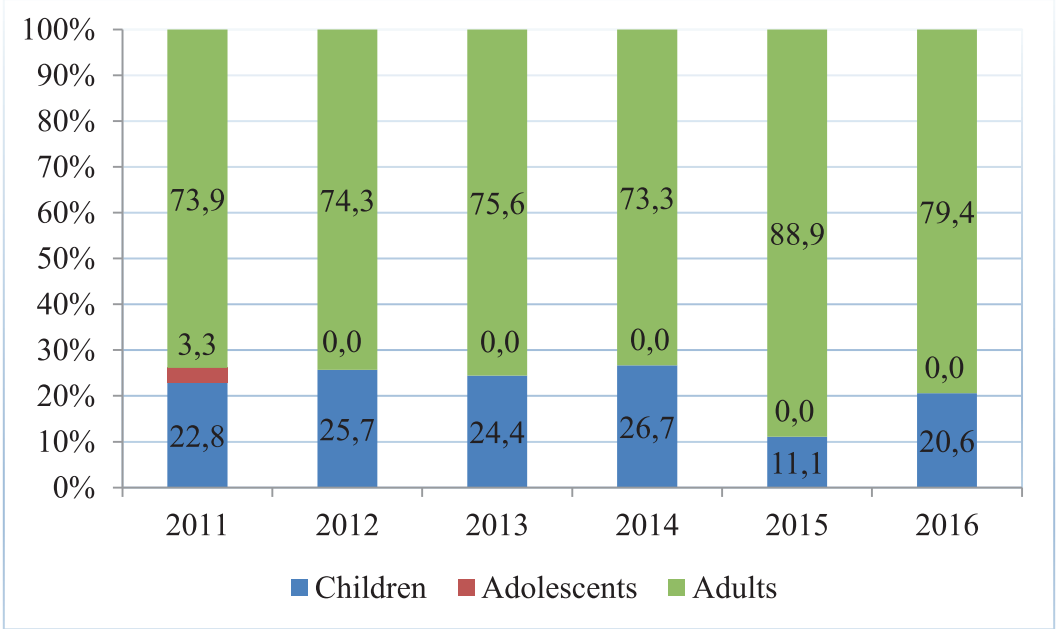

Figure 3. Specific weight of patients with acute otitis media by age groups in Altai Krai for the period of 2011-2016 (at percentage)

Table 1

The etiological structure of bacterial meningitis in Altai Krai for the period of 2014-2016

Year	Samples (pcs)	Positive result (pcs)	Among them:				
			N. meningitidis				Str. Pneumoniae
			Gr. A	Gr. B	Gr. C	Total	
2014	6	6	-	3	1	4	2
2015	23	8	-	3	-	3	5
2016	24	15	-	3	3	6	9
Total	53 (100%)	29 (54,7%)	13 (44,8%)				16 (55,2%)

period from 2015 to 2016 the morbidity rate increased by 27.3 per cent. The highest BM morbidity rate was among children and amounted to 2.9±0.35 per 100 thousand of the corresponding population. Adult population made the main contribution to the structure of patients with BM, the specific weight equaled to 76.2±2.45 per cent. Pneumococcal disease took the leading position in BM etiology in the area for the period under study. In Altai Krai, there is no pneumococcal disease epidemiological surveillance system. These results confirmed the necessity of its development and implementation in the territory of a large area.

References

1. Lobzin Yu.V. et al. Serotypes of Streptococcus pneumoniae causing major pneumococcal infections. *Journal Infectology*. 2013; 5(4): 36-42.

2. Kharit S.M., Perova A.L. Modern approaches to the prevention of pneumococcal infection. *Meditsinsky sovet*. 2015; 16: 64-67.

3. O'Brien K.L. et al. Burden of disease caused by Streptococcus pneumoniae in children younger than 5 years: global estimates. *Lancet*. 2009; 374: 893-902.

4. Sakai F. et al. Single-plex quantitative assays for the detection and quantification of most pneumococcal serotypes. *PloS one*. 2015; 10(3): 47-54.

Contacts

Corresponding author: Kozyanova Yulia Anatolyevna, post-graduate student of the Department of epidemiology, microbiology and virology of ASMU, Barnaul.
656056, Barnaul, ul. Papanintsev, 126.
Tel.: (3852) 566924.
E-mail: ykozyanova@yandex.ru

UDC 616.62-003.7-092.4

PHARMACOLOGICAL MODULATION OF THE EXPRESSION OF INTRARENAL INHIBITORS OF CRYSTALLIZATION AND OXIDATIVE STRESS MARKERS OF NEPHROCYTES BY EXPERIMENTAL KIDNEY STONE DISEASE

Altai State Medical University, Barnaul

A.Yu. Zharikov, N.N. Yakushev

The present research is aimed at the study of sodium citrate and α-tocopherol acetate effect on the level of expression of Tamm-Horsfall protein, osteopontin, bikunin, malondialdehyde and superoxide dismutase in the kidney tissue of rats by experimental kidney stone disease.

The experiments were performed in 60 male Wistal rats at the age of 2-3 months and 250-300 g of weight. The animals were equally divided into 4 groups: the group of intact rats – $G_{int.}$ (42 days in standard laboratory conditions without KSD modelling and without injection of the studied drugs), control group – G_c (42 days of KSD modelling), group of treatment by sodium citrate – $G_{n.c.}$ (42 days of KSD modelling + daily injection of sodium citrate from 22nd to 42nd day of experiment), group of treatment by α-tocopherol acetate – $G_{t.a.}$ (42 days of KSD modelling + daily injection of α-tocopherol acetate from 22nd to 42nd day of experiment). The experimental kidney stone disease was modelled in accordance with the generally accepted ethyleneglycol model. The expression of Tamm-Horsfall protein, osteopontin, bikunin, superoxide dismutase and malondialdehyde was determined by means of indirect two-stage streptavidin-biotin method with the control of reaction specificity.

It was determined, that the expression of Tamm-Horsfall protein, osteopontin, bikunin, superoxide dismutase and malondialdehyde in conditions of KSD modelling was exposed to considerable alterations in relation to the healthy rats. On this background, long-term continuous injection of sodium citrate and α-tocopherol acetate was accompanied by the normalization of expression of intrarenal inhibitors of crystallization and oxidative stress markers of nephrocytes.

Key words: *kidney stone disease, intrarenal inhibitors of crystallization, oxidative stress markers, sodium citrate, α-tocopherol acetate.*

Introduction

According to modern concepts, the qualitative and quantitative changes in the expression of intrarenal crystallization inhibitors, such as the Tamm-Horsfall protein (THP), osteopontin (OPN) and bikunin (BIK), play a crucial role in the process of stone formation by kidney stone disease (KSD), as a result of which they lose their inhibitory properties or even become stimulants of lithogenesis [1-4]. In addition, the destructive role of the process of free radical oxidation (FRO) by KSD, which leads to the formation of a primary source of lithogenesis with subsequent precipitation of the crystalline material from urine [5-7], is not subject to doubt. Histochemical markers of the FRO process can be the level of expression of malonic dialdehyde (MDA) and superoxide dismutase (SOD) in kidneys, whose role in activation and inhibition of FRO is well known [5-7].

In this connection, changes in the parameters of the intraluminal expression of mentioned substances can become an important indicator of the effectiveness of a method of pharmacological correction of urolithiasis. Given this circumstance, we were interested in the possibility of evaluating the effect of some known antilithogenic agents on the level of expression of intrarenal crystallization inhibitors and markers of oxidative stress in the kidney tissue.

Research objective

To study the effect of sodium citrate and α-tocopherol acetate on the level of expression of the Tamm-Horsfall protein, osteopontin, bikunin, malonic dialdehyde and superoxide dismutase in rat kidney tissue by experimental kidney stone disease.

Materials and methods

The experiments were carried out in 60 male Wistar rats aged 2-3 months and weighing 250-300 grams grown in the nursery of the Research Institute of Cytology and Genetics of the SB RAS (Novosibirsk). The studies were carried out in accordance with the principles of humanity set out in the directives of the European Community (86/609 / EEC) and the Helsinki Declaration, in accordance with the "Rules for Working with Experimental Animals". The animals were divided equally into 4 groups: a group of intact rats - G_{int} (42 days of maintenance in standard laboratory conditions without KSD modelling and without the administration of studied drugs), control group G_c (42 days of KSD modeling), group of sodium citrate treatment – $G_{s.c.}$ (42 days of KSD modeling + daily administration of sodium citrate from 22nd to 42nd days of the experiment), group of α-tocopherol acetate treatment – $G_{t.a.}$ (42 days of KSD modeling +

daily administration of α-tocopherol acetate from the 22nd to 42nd days of the experiment).

Experimental kidney stone disease was modeled in accordance with the generally accepted ethylene glycol model, according to which animals were given a 1% solution of ethylene glycol on a daily basis as a drink [8]. α-tocopherol acetate is a classic antioxidant used in the treatment of KSD [7]. The drug was injected through the probe in the form of an oily solution at a dose of 300 mg/kg. Sodium citrate is a drug that has the ability to chelate lithogenic ions and/or their insoluble salts in the urine [9]. Was injected through the probe in a dose of 200 mg / kg.

At the end of 6 weeks of the experiment, the rats were decapitated under complete ether anesthesia, after which kidneys were removed to conduct an immunohistochemical study.

Determination of the expression of the Tamm-Horsfall protein, osteopontin and bikunin, as well as superoxide dismutase and malonic dialdehyde, was carried out with the help of an indirect two-step streptavidin-biotin method with control of the specificity of the reaction [10]. After the standard procedure of dewaxing and rehydration, endogenous peroxidase was blocked according to the recommendations of the antibody manufacturer (Santa Cruz, USA).

Morphometric studies were carried out using the software packages ImageJ 1.43 and AxioVision 3.1. The degree of expression was evaluated by a semi-quantitative method using the σ^2 criterion for the intensity of DAB staining using ImageJ 1.43 image analysis software. For the convenience of interpreting the results, the obtained data were calculated by the formula:

$$E\% = 100 - \frac{100 \times D}{256}x;$$

Where: E% – percent of expression;

256 – maximum color intensity of the cutoff field.

Results and discussion

As a result of the conducted experiments, it was found that in the G_{int} rats, the expression of the Tamm-Horsfall protein was noted mainly in the cytoplasm and on the apical membranes of epithelial cells of the distal tubules of the cortex and the thick ascending section of the Henle loop of the outer zone of the medulla. Expression of osteopontin was recorded mainly in the cytoplasm of the epitheliocytes of the nephron tubules, collecting tubes, transitional epithelium of the cup-and-pelvis system. In addition, moderate bikunin expression of cytoplasmic localization in the epithelium of the nephron tubules and collecting tubes and the absence of such in the interstitium were observed. The quantitative indices of the expression of THP, OPN, BIK and also MDA and SOD are presented in Table 1.

Against this background, under the conditions of KSD modeling, significant changes in the qualitative and quantitative indices of the expression of THP, OPN, BIK, MDA and SOD were observed in the control group. It turned out that the expression of THP in the interstitium of the renal papilla, which was most pronounced in the region of the base and middle third, was also noted, i.e. in places where deposits of calcium predominate. After 6 weeks of ethylene glycol implementation, the degree of expression of THP in relation to intact rats was significantly increased by 4.6% (Table 1). There was no topological difference in the expression of arterial hypertension with respect to intact animals in the control of the disease. However, by the long-term use of ethylene glycol, the expression of OPN was weakened by 3.2% compared to that of intact animals (Table 1). In the kidneys of the rats in the control group with similar overall localization, the BIK expression was also detected in the interstitium of the renal papilla, i.e. in the place of preferential deposition of calcium deposits. At the same time, the level of BIK expression in quantitative terms slightly exceeded the indices of the intact group (Table 1).

The level of MDA expression in points in the $G_{c.}$ group increased from 2+ in norm to 3+ (Table 1). In percent, the recorded increase constituted 3.5%. Against this background, the level of SOD expression, on the contrary, declined. So, if in intact rats this indicator was estimated as 3+, then after six weeks of modeling the disease, it was reduced to 2+. In quantitative terms, the regression of the degree of SOD expression constituted 5.8%.

The experiments conducted in the $G_{s.c.}$ group showed that as a result of the course of sodium citrate implementation, the expression of inhibitors of crystallization was normalized. Thus, the topology of the expression of THP, the signs of which were detected in the interstitium of the renal papilla in the control of the disease, i.e. in the place of preferential deposition of calcium deposits, in the rats treat with sodium citrate completely corresponded to the intact group: cytoplasm and apical membranes of the epithelial cells of the distal tubules of the cortex and the thick ascending section of the Henle loop of the outer zone of the medulla without signs of interstitial localization. At the same time, there was no topological difference in the expression of OPN and BIK between the groups.

Despite this, the quantitative characteristics of the expression of all the proteins described by the results of the course of treatment underwent significant changes. As follows from Table 1, the level of THP expression decreased relative to control from 3+ points to 2+ points, which in arithmetic interpretation constituted 3.2%. Expression of acute renal failure, on the contrary, increased from 2+

points to 3+ points, i.e. by 2.5%, and the expression of BIK - weakened by 3.4%.

Thus, the pattern of expression of intrarenal crystallization inhibitors as a result of a three-week administration of sodium citrate was generally consistent with that of healthy animals.

The study of the effect of long-term administration of α-tocopherol acetate on the expression of intrarenal crystallization inhibitors and markers of oxidative damage to nephrocytes made it possible to establish that the studied drug leads to a characteristic change in the expression of THP, OPN, and BIK. It turned out that expression of THP in the kidneys of $G_{t.a.}$ rats was detected in the cytoplasm and on the apical membranes of epithelial cells of the distal tubules and the thick ascending section of the Henle loop of the outer zone of the medulla, which fully corresponded to the topology of intact rats. It is important that there was no interstitial localization of the THP expression as a result of the treatment, although it was fixed in the control of the disease, which was a characteristic immunohistochemical sign of the pathology. There was no visual topological difference in the expression of OPN between the groups. With respect to BIK expression, it was found that in the kidneys of $G_{t.a.}$ rats, it is present in the epithelium of the nephron tubules and collecting tubes, and there was no interstitial localization in the region of the renal papilla that took place in the control of the disease.

A quantitative assessment of the changes in the expression of the above proteins, as shown in Table 1, revealed a decrease in the expression of the THP from 3+ points in the control to 2+ points in the rat kidneys of the $G_{t.a}$ group, which in the percentage measurement was 3.3%. In parallel with the 1+ points in the control, up to 2+ points after the course of α-tocopherol acetate (by 4.4%), the expression of OPN increased, as well as from 2+ points in sick animals to 1+ points in rats with long-term α-tocopherol acetate administration, the degree of expression of BIK decreased. In percent, this decline was 4.1%. The recorded changes led to the fact that the level of expression of THP and OPN after the course of antioxidant therapy corresponded to that of healthy animals, and the same indicator for BIK was even inferior to intact figures of 2.7%.

Moreover, in $G_{t.a.}$ rats, the level of MDA expression relative to disease control decreased from 3+ points to 2+ points, which in quantitative terms was 4.1% (Table 1). The same indicator for SOD increased from 2+ points in the control to 3+ points in the treatment group, which was 5.7%. Such changes were realized in the fact that the degree of MDA expression corresponded exactly to the value of healthy rats, and the expression of SOD even exceeded that of 2.2% (Table 1).

Thus, continuous administration of α-tocopherol acetate was accompanied by normalization of expression of intrarenal crystallization inhibitors and markers of oxidative damage to nephrocytes.

Summarizing the foregoing, we note that the qualitative and quantitative characteristics of the expression of THP, OPN, BIK, MDA and SOD under the conditions of KSD modeling were subject to significant changes with respect to healthy rats. These changes could be both pathological and compensatory. Nevertheless, in both cases, they can be recognized as markers for the development of the kidney stone disease. In this case, the three-week administration of sodium citrate and α-tocopherol acetate resulted in normalization of the expression indices of the above substances. As is known, sodium citrate is a chelating agent, and α-tocopherol acetate is a direct non-enzyme antioxidant [7,9]. These substances have now been proven to possess antilithogenic properties. Both drugs affect the main driving forces of crystallization: sodium citrate - to supersaturation of urine with calcium ions, α-tocopherol acetate - to oxidative damage to nephrothelia [7,9]. It is not excluded that these substances, targeting the proper links of the pathogenesis of nephrolithiasis, significantly weakened the severity of the pathology, which neutralized the conditions for changes in the pattern of expression of THP, OPN, BIK, MDA and SOD, bringing it back to normal. Of course, the features of the modulation of the expression of nephrolithiasis markers by the KSD and in the conditions of its treatment require a more careful study. However, based on the results of this study, with some confidence, it can be assumed that qualitative and quantitative changes in the expression of the mentioned proteins can be a very informative indicator for evaluating the effectiveness of pharmacological treatment of urolithiasis.

Conclusion

The three-week administration of sodium citrate and α-tocopherol acetate by experimental kidney stone disease leads to normalization of the qualitative and quantitative parameters of the expression of THP, OPN, BIK, MDA and SOD significantly altered under the conditions of the disease.

References

1. Zverev Ya.F., Zharikov A.Yu., Bryukhanov V.M., Lampatov V.V. Modulators of oxalate nephrolithiasis. Inhibitors' crystallization. *Nephrology.* 2010. 1 (14): 29-49.

2. Garimella PS, Sarnak MJ. Uromodulin in kidney health and disease. *Curr Opin Nephrol Hypertens*. 2017. 2 (Vol. 2): 136-142.

3. Yasui T, Okada A, Hamamoto S et. al. Pathophysiology-based treatment of urolithiasis. *Int J Urol*. 2017. 1 (Vol. 24): 32-38.

Table 1

Indices of expression of intrarenal crystallization inhibitors and markers of oxidative stress in renal tissue

KSD markers	Groups of animals							
	G_{int}		G_c		$G_{s.c.}$		$G_{t.a.}$	
	Rate of expression							
	Points	%	Points	%	Points	%	Points	%
Tamm-Horsfall protein	2+	49,8	3+	54,4	2+	51,2	2+	51,1
Osteopontin	2+	50,0	1+	46,8	2+	49,3	2+	51,2
Bikunin	2+	50,6	2+	52,0	2+	48,6	1+	47,9
Malondialdehyde	2+	48,0	3+	51,5	2+	47,6	2+	47,4
Superoxide dismutase	3+	52,5	2+	46,8	3+	51,0	3+	54,7

4. Igci M, Arslan A, Igci YZ et. al. Bikunin and α1-microglobulin/bikunin precursor (AMBP) gene mutational screening in patients with kidney stones: a case-control study. *Scand J Urol Nephrol*. 2010. 6 (Vol. 44): 413-419.

5. Zharikov A.Yu., Bryukhanov V.M., Zverev Ya.F. et al. Oxidative stress as one of the factors of damage in the early stages of experimental nephrolithiasis. *Morphology*. 2011. 1 (5): C. 33-37.

6. Motina N.V., Zverev Ya.F., Lepilov A.V. et al. Oxidative renal damage in experimental oxalate nephrolithiasis. *Nephrology*. 2010. 1 (14): 68-72.

7. Zharikov A.Yu., Talalayeva O.S., Zverev Ya.F. et al. The role of antioxidant therapy in the pharmacological correction of experimental nephrolithiasis. *Nephrology*. 2010. 4 (14): 53-58.

8. Bryukhanov V.M., Zverev Ya.F., Lampatov V.V., Zharikov A.Yu. Methodical approaches to the study of kidney function in an animal experiment. *Nephrology*. 2009. 3 (13): 52-62.

9. Marangella M. Use of citrate in patients with nephrolithiasis. *G Ital Nefrol*. 2017. 4 (Vol. 34): 51-60.

10. Gurevich L.Ye., Isakov V.A. Use in immunohistochemical studies of the method of restoration of antigenic specificity by the action of microwaves on tissues fixed with formalin and encapsulated in paraffin. *Archive of pathology*. 1999. 2: 48-50.

Contacts

Corresponding author: Zharikov Aleksandr Yuryevich, Doctor of Biological Sciences, Associate Professor, Head of the Department of Pharmacology of ASMU, Barnaul.

656056, Barnaul, ul. Papanintsev, 126.

Tel.: (3852) 24-18-59, 56-68-91.

E-mail: zharikov@agmu.ru

UDC 615.27-092.4

COMPARATIVE ASSESSMENT OF HISTOCHROME'S ANTIOXIDANT AND PROOXIDANT ACTIVITIES IN TERMS OF *IN VITRO* AND *IN VIVO* EXPERIMENTS

Altai State Medical University, Barnaul

O.S. Talalayeva, Ya.F. Zverev, S.V. Zamyatina

The current study presents the results of comparison of antioxidant and pro-oxidant histochrome activity in terms of in vitro and in vivo experiments. In vitro effect of two concentrations of the drug approximately corresponding to the doses administered by animals was studied. In vivo experiments were performed on Wistar rats, randomized into three groups: the 1st and 2nd groups received histochrome at a dose of 1 mg/kg (n = 15) and 10 mg/kg (n = 15), the 3rd one was the control group. After 14 days of drug injection, oxidative stress was induced and the total antioxidant activity (TAA) and total pro-oxidant activity (TPA) in the rat blood were evaluated.

In terms of study it was revealed, that histochrome can have multidirectional effect on the processes of free-radical oxidation in model systems in vitro and in vivo. The prevalence of antioxidant activity was registered by the injection of the drug at a dose of 1 mg/mk.

***Key words**: histochrome, antioxidant and pro-oxidant properties.*

Histochrome is a water-soluble dosage form of an individual substance, a natural quinoid pigment of marine invertebrates of echinochrome A (2,3,5,6,8-pentahydroxy-7-ethyl-1,4-naphthoquinone) [1]. The basis for the development of drugs of the "Histochrome" series (state registration number 002363/01-2003 and 002363/02-2003) was the high pluripotent antioxidant activity of echinochrome A [2,3,4].

The first data on the expressed reactivity of 2,3,5,6,8-pentahydroxy-7-ethyl-1,4-naphthoquinone were obtained by the workers of the G.B. Elyakov Pacific Institute of Bioorganic Chemistry in *in vitro* experiments. In various model systems, it has been shown that the donor properties of echinochrome A allow it to restore lipid radicals, ensuring the structural and functional integrity of cytoplasmic membranes [5,6]. The ability of 2,3,5,6,8-pentahydroxy-7-ethyl-1,4-naphthoquinone to neutralize superoxide aniline has been proved by competitive inhibition of the reduction of nitro blue tetrazolium [6]. The opinion was also expressed that the antioxidant potential of 2,3,5,6,8-pentahydroxy-7-ethyl-1,4-naphthoquinone is largely due to its ability to chelate iron cations, which participate in the branching of chain radical reactions [5,7,8,9,10].

Thus, in numerous model systems and chemical tests, a high polytarget antiradical activity of echinochrome A was established.

At the same time, it is known that the antioxidant activity of the substance detected *in vitro* should not be fully extrapolated to *in vivo* conditions, since under the conditions of a living organism, one and the same substances can simultaneously manifest both antioxidant and prooxidant activity [11,12]. In principle, the ability of histochrome to have a multidirectional effect in terms of *in vitro* and *in vivo* experiments was previously shown by us in the study of the membrane stabilizing effect of the drug [13].

Research objective

Thus, the objective of the current study is a comparative evaluation of the antioxidant and prooxidant activities of histochrome drug in *in vitro* and *in vivo* experiments.

Materials and methods

In the experiment, there was used the "Histochrome® solution for intravenous injection of 1% in 5 ml ampoules" (registration number P N002363 / 01-2003 dated 07.23.2008) (PIBOC FEB RAS, Vladivostok) [1].

Experiments *in vivo* were performed in outbred Wistar rats of both genders aged 2-3 months and weighing 200-300 g grown in the breeding nursery of the Research Institute of Cytology and Genetics of the SB RAS (Novosibirsk). The animals were kept in standard conditions in a vivarium on a full balanced diet in accordance with the recommendations of the Institute of Nutrition, RAMS, in a well-ventilated room with a temperature of + 20 ± 2 °C and a moisture content of not more than 80%. The experiments were performed in the spring period from 9.00 to 15.00. Randomization of animals into three groups was performed by a block method. The first two groups of rats daily received histochrome at a dose of 1 mg/kg (experimental group 1, n = 15) and 10 mg/kg (experimental group 2, n = 15) for 14 days subcutaneously. The third group of animals was the control one and received equivolume subcutaneous injections of isotonic sodium chloride solution (n = 15).

At the end of the course, exudative inflammation was modeled. For this, 0.2 ml of a 3% forma-

lin solution was injected into the plantar aponeurosis of both posterior limbs of rats. Earlier in our laboratory, it was shown that subplantal injection of formalin is accompanied by the development of oxidative stress with a peak in two days after the injection of phlogistic [14]. On the third day of exudative inflammation, rats were decapitated under ether anesthesia and the indices of activity of free radical oxidation processes were determined in the blood. The results obtained were compared with the data specific for intact animals. The total prooxidant activity (TPA), the integrative index of the concentration of all prooxidants and the activity of the processes of lipid peroxidation were evaluated by the ability of the blood plasma to induce the oxidation of TWEEN-80 with further photocolorimetric determination of the content of products reacting with thiobarbituric acid (TBRP, the results are presented as a percentage of the value of the control sample) [15].

Antioxidant activity in erythrocyte hemolysate was assessed by the change in the integrative index of total antioxidant activity (TAA, determined by inhibition of the Fe^{2+}/ascorbate induced accumulation of TBRP during the oxidation of TWEEN-80, the results are expressed as a percentage of the value of the control sample) [16,17,18]. The described system simulates the peroxidation of plasma lipids [19].

All *in vivo* experiments were carried out in accordance with the requirements of the European Convention for the Protection of Vertebrates Used for Experimental or Other Scientific Purposes (Strasbourg, 1986) and the Federal Law of the Russian Federation "On the Protection of Animals against Cruel Treatment" of 01.01.1997.

In two series of *in vitro* experiments, antioxidant and prooxidant activity of histochrome was evaluated [15,16,18]. To determine the total prooxidant (TPA) and total antioxidant activity (TAA), instead of the biological material, a corresponding volume of a solution of histochrome in the studied concentration was introduced into the test tubes (Table 1).

The peculiarity of the study was the use of patented model systems (ASMU, the Department of Biochemistry and Clinical Laboratory Diagnostics) maximally close to the conditions of the whole organism. Both in *in vitro* and *in vivo* experiments, evaluation of the antioxidant and prooxidant activity of the histochrome drug was performed using a single technique, which allowed to maximize the objectivity of the results of the comparative analysis. Analyzed drug concentrations were equivalent to histochrome doses used in two groups of rats. The methodologies were validated.

Table 1

Design of in vitro experiments

Test type	Test number, *n*	Initial concentrations of studied histochrome solutions	
Prooxidant activity of histochrome			
Control tests	20	Distilled water, 0,2 ml	
Experimental tests	20	0,1 mg/ml, 0,2 ml	0,2 mg/ml, 0,2 ml
Antioxidant activities of histochrome			
Control tests	20	Distilled water, 0,1 ml	
Experimental tests	20	0,1 mg/ml, 0,1 ml	0,2 mg/ml, 0,1 ml

The results were processed by means of "Statistica for Windows 6.0" software package. The results are presented in the form of M ± m, where M is the sample mean, m is the error of the mean, n is the sample. The dynamics of the indices in the dependent samples of pair measurements was carried out with the help of the Wilcoxon test. For the intergroup estimation of nonparametric indices of independent samples, the Mann-Whitney test was used. The level of statistical significance corresponded to $p < 0.05$.

Results

The study of the antioxidant activity of histochrome in *in vivo* experiments was carried out on the model of pathology accompanied by oxidative stress caused by the plantar injection of formalin followed by the formation of inflammatory edema of posterior limbs of the rats [14,20]. Preliminary, to determine the optimal time for the evaluation of antioxidant activity, the dynamics of oxidative stress development against the background of formalin edema was studied (Table 2).

Considering the dynamics of oxidative stress caused by the inflammatory process, the determination of antioxidant and prooxidant activity in *in vivo* experiments was performed at the peak of formalin edema reaching its maximum on the third day after the injection of the phlogogen.

Table 2

Effect of prolonged injection of various doses of histochrome on the activity of free radical oxidation processes in rat blood

Index	Intact rats	Control rats	Histochrome	
			1 mg/kg	10 mg/kg
Total prooxidant activity, %	45,1 ± 1,06	60,1 ± 1,25*	23,7 ± 1,24*,#	61,1 ± 1,16*
Total antioxidant activity, %	73,7 ± 0,51	87,8 ± 0,86*	38,9 ± 2,01*,#	17,4 ± 0,70*,#

Note: * - statistically significant difference from the corresponding index in intact rats, # - significant difference from the corresponding index in control rats.

As in the case of *in vitro* experiments, the increase in free radical derivatives in the blood plasma of animals was characterized by a linear dependence on the amount of the active principle of histochrome (Tables 2, 3). It should be noted that in the group of animals receiving histochrome at a dose of 1 mg/kg, the TPA value was 2.5 times lower than the values characterizing oxidative stress in animals with experimental edema, and half as much as in intact animals (Table 2). A tenfold increase in the dose of the drug neutralized the registered effect, and the concentration of free radical derivatives in the plasma of the experimental rats corresponded to that in the control group of animals under conditions of maximum activity of oxidative stress.

As shown in Table 2, an inverse relationship was recorded for antioxidant activity and dose of the drug. In conditions of prolonged injection of histochrome in rats with experimental inflammation, a dose-dependent but multidirectional drug effect was detected. Thus, the injection of 1 mg/kg histochrome in animals with experimental inflammation led to suppression of the processes of lipid peroxidation and a tenfold increase in the dose to activation.

The data of the *in vitro* experiments presented in Table 3 show a concentration-dependent increase in both prooxidant and antioxidant activities. At the same time, the increase in TPA prevailed at all concentrations used.

Table 3

Indexes of prooxidant and antioxidant activities of various concentrations of histochrome in in vitro experiments

Index	Studied histochrome concentrations	
	0,1 mg/ml, *n = 20*	0,2 mg/ml, *n = 20*
TPA (%)	15,5 ±1,45	20,3 ±1,78, p = 0,053
TAA (%)	9,4 ± 1,20, $p^* = 0,003$	12,2 ±1,24, $p^* = 0,000$

Note: *p* - statistically significant differences in effects between the concentrations studied, *p** - statistically significant differences between the corresponding concentrations of TAA and TPA.

Discussion

The results of the study detected differences in the nature of the changes in processes of free radical oxidation in *in vivo* and *in vitro* experiments.

The analysis of the prooxidant activity of histochrome in *in vivo* experiments showed that when the dose equivalent to the therapeutic dose of a human was injected into the animals for a prolonged period, the drug, by lowering the level of free-radical derivatives in the rat blood plasma, manifested itself as an antioxidant. At a dose of 10 mg/kg, according to the TPA data, histochrome did not possess such an effect. In parallel, the evaluation of antioxidant activity in *in vivo* experiments showed that the increase of the dose of the drug is accompanied by a decrease in its protective properties. At the same time, in *in vitro* experiments, the increase in prooxidant activity prevailed in all analyzed concentrations of the drug.

The most logical explanation of the differences in the results obtained in *in vivo* and *in vitro* experiments is the involvement of certain additional mechanisms in the action of histochrome, induced in a holistic organism. Modern studies have shown fundamental differences in the activity of phenolic antioxidants in chemical tests and in the cellular system resulting from the involvement of endogenous mechanisms [12,21]. It is known, for example, that many polyphenolic compounds are capable of modifying the activity of endogenous antioxidant systems [20, 21, 22], secondary intracellular messengers and possess genomic effects [21, 23]. Specifically, the presence of genomic effects was established in echinochrome A [24, 25, 26]. Among

the latter, special attention is paid to the ability of this naphthoquinone to modulate the activity of the p53 gene [26].

At the same time, it should be noted that, in spite of the significant antioxidant capacity, under certain conditions, polyphenols can exhibit prooxidant properties [12, 27]. It is natural that with the increase of the amount of active substance, the prooxidant activity of the drug increases. The obtained results to a certain extent explain the data of the quantum-chemical analysis of the structural basis of histochrome-2,3,5,6,8-pentahydroxy-7-ethyl-1,4-naphthoquinone. It can be assumed that prolonged injection of high doses in animals with experimental inflammation was accompanied by excessive production of the reactive radical naphthoquinone and hydrogen peroxide, which in turn supported the activity of lipid peroxidation at a high level [27,28]. In this case, it becomes clear why the increase in the dose of histochrome to 10 mg/kg was accompanied by the activation of the free-radical oxidation processes. In turn, the antioxidant effect of histochrome manifested itself only in the absence of its prooxidant activity, which was recorded by the use of smaller doses of the drug.

The protective effect of histochrome in relation to the processes of free radical oxidation is most likely determined not by the minor mechanism, but by the ratio of the various antioxidant effects of the drug. Histochrome realizes antioxidant activity in doses equivalent to the therapeutic dose of a human, and a tenfold increase in the latter is accompanied by an increase in the prooxidant activity of the drug.

Conclusions

The results of the study showed a predominance of prooxidant activity of histochrome in *in vitro* experiments.

The antioxidant/prooxidant activity ratio of the drug in *in vivo* experiments was positive when a dose equivalent to the therapeutic dose of a human was injected. A ten-fold increase in the dose of histochrome transformed the ratio toward the activation of free radical oxidation processes.

The multidirectional action of Histochrome® drugs on biological processes in model systems *in vitro* and *in vivo* indicates the presence of endogenous mechanisms in echinochrome.

References

1. State Register of Medicines. Registration certificate. Available at: http://grls.rosminzdrav.ru/Grls_View_v2.aspx?routingGuid=39892aad-5327-4a6c-99d5-2dc5a51ee2e7&t=

2. RF Patent No. 2134107/10.08.1999. Yelyakov G.B., Maksimov O.B. Medicinal preparation "Histochrome" for the treatment of inflammatory diseases of the retina and the cornea of the eyes.

3. RF Patent No. 2137472/20.09.1999. Yelyakov G.B. Medicinal preparation "Histochrome" for the treatment of acute myocardial infarction and ischemic heart disease.

4. Khabriev R.U. State Register of Medicines, "Scientific Center for Expert Evaluation of Medical Applications" Moscow: Ministry of Health of Russia; 2004.

5. Mischenko N.P., Prokofieva N.G., Fedoreyev S.A. Antiradical and hemolytic activity of quinoid pigments of sea urchins. Research in the field of physico-chemical biology and biotechnology. Theses of the reports of the regional scientific conference. Vladivostok, 2004.

6. Lebedev A.V., Ivanova M.V., Krasnovid N.I., Koltzova E.A. Weak acid properties of hydroxylated naphthazarins and their reaction with superoxide anion-radical. *Biomeditsinskaya Khimiya*. 1999; 45(2): 123-130.

7. Takhchidi Kh.P., Metaev SA, Kagirov R.R. Antioxidant protection of the retina in experimental hemophthalmia in rabbits. *Ophthalmic surgery*. 2003; 2: 14-16.

8. Priezzhaeva E.Yu., Lebedko O.A., Ryzhavsky B.Ya. et al. Effect of echinochrome A on the structure and metabolism of the kidneys of 40-day-old white rats subjected to prenatal exposure to lead nitrate. *Pacific Medical Journal.* 2009; 3: 58-60.

9. Lebedev A.V., Ivanova M.V., Levitsky D.O. Echinochrome, a naturally occurring iron chelator and free radical scavenger in artificial and natural membrane systems. *Life Sci.* 2005; 76(8): 863-875.

10. Lebedev A.V., Ivanova M.V., Levitsky D.O. Iron chelators and free radical scavengers in naturally occurring polyhydroxylated 1,4-naphthoquinones. *Hemoglobin*. 2008; 32(1): 165-179.

11. Ratkin Ye.V., Ivanov V.V., Ratkin A.V. Antioxidant and antiradical properties of polyphenols in the mechanism of hepatoprotective action of the preparations of the Amur maakia. *Bulletin of Siberian Medicine.* 2011; 5: 91-94.

12. Bizunok N.A. The antioxidant activity structure determinants of the phenol, diphenol and polyphenols derivatives in regard to ros, generated by macrophages in various microenvirons. *Military medicine: scientific and practical peer-reviewed journal.* \2013; 1: 84-94.

13. Talalayeva O.S., Zverev Ya.F., Zamyatin S.V., Bryukhanov V.M., Lampatov V.V. The effect of histochrome on the osmotic resistance of red blood cells in the in vitro and in vivo experiments. *Siberian medical journal.* 2012; 27(4): 70-74.

14. Tikhomirova S.V., Bryukhanov V.M., Zverev Ya.F. Antioxidant effect of collection of medicinal plants used in experimental glomerulonephritis. *Nephrology.* 2004; 8(2): 155-156.

15. RF Patent No. 2146053/10.02.1997. Molchanov A.V., Galaktionova L.P. A method for determining the prooxidant activity of a biological material.

16. Blagorodov S.G., Shepelev A.P., Dmitriyeva N.A. et al. Determination of the antioxidant activity of chemical compounds. *Pharmaceutical Chemistry Journal.*

1987; 21(3): 292-294.

17. Galaktionova L.P. Peculiarities of oxidant-antioxidant status change in patients with bronchial asthma with drug and non-medical correction. [synopsis of a thesis]. Novosibirsk, 2004.

18. Kolenchenko Ye. A., Sonina L.N., Hotimchenko Yu.S. Comparative evaluation of the antioxidant activity of low esterified pectin from the Zostera Marina sea herb and antioxidant preparations in vitro. *Russian Journal of Marine Biology.* 2005; 31(5): 380-383.

19. Opeida I.A., Shendrik A.N., Kachurin I.O. Kinetics of oxygen absorption and chemiluminescence in the oxidation of lipids in the presence of Fe2 + ions. *Kinetics and catalysis.*

20. Talalayeva O.S., Mishchenko N.P., Bryukhanov V.M. et al. Effect of histochrome on the process of free radical oxidation in the experiment. *Bull. SB RAMS.* 2011; 31(3): 63-67.

21. Ching-Hsein C., Miao-Ling L., Ping-Lin O. et al. Novel multiple apoptotic mechanism of shikonin in human glioma cells. *Ann. Surg. Oncol*. 2012; 19(9): 3079-3106.

22. Artiukov A.A., Popov A.M., Tsybul'skii A.V. et al. Pharmacological activity echinochrome A singly and consisting of BAA "Timarin". *Biomed. Khim*. 2012; 58(3): 281-290.

23. Bharathi R.S., Gayathri S., Sakeena S.M.S. et al. Apoptosis inducing effect of plumbagin on colonic cancer cells depend on expression of COX-2. *PLos ONE*. 2011; 6(4): 1-11.

24. Eremenko E.M., Antimonova O.I., Shekalova O. et al. Novel compounds that increase expression of Hsp70 and its biological activity. *Cell and Tissue Biology*. 2010; 4(3): 251–257.

25. Lennikov A., Kitaichi N., Noda K. et al. Amelioration of endotoxin-induced uveitis treated with the sea urchin pigment echinochrome in rats. *Mol. Vis.* 2014; 20: 171-177.

26. Kareyeva Ye.N., Tikhonov D.A., Mischenko N.P., Fedoreyev S.A. Influence of histochrome on p53 expression in red bone marrow cells of mice under the conditions of a model of chronic stress. *Pharmaceutical Chemistry Journal.* 2014; 48(3): 9-12.

27. Lebedev A.V., Levitskaya E.L., Tichonova E.V. et al. Antioxidant properties, autooxidation, and mutagenic activity of echinochrome A compared with its etherified derivative. *Biochemistry (Mosc).* 2001; 66(8): 885-893.

28. Berdyshev D.V., Glazunov V.P., Novikov V.L. Study of the mechanisms of antioxidant activity of 2,3,5,6,8-pentahydroxy-7-ethyl-1,4-naphthoquinone (echinochrome) using the theory of functional density. Communication 1. The interaction of echinochrome A with a hydroperoxide radical. *Russian Chemical Bulletin.* 2007; 3: 400–415.

29. Yankova V.I., Knyshova V.V., Lankin V.Z. Oxidative stress control in cases of alimentary dyslipidemia by antioxidants produced from marine hydrobionts. *Bull. SB RAMS.* 2010; 30(1): 64–69.

30. Lebedkova O.A., Ryzhavsky B.Ya., Demidova O.V. Effect of the antioxidant echinochrome A on bleomycin-induced pneumofibrosis. *Bulletin of Experimental Biology and Medicine.* 2015; 159(3): 351-354.

31. Klochkov S.G., Neganova M.Ye., Afanasyeva S.V., Shevtsova Ye.F. Synthesis and antioxidant activity of securinin derivatives. *Pharmaceutical Chemistry Journal*. 2014; 48(1): 18-21.

Contacts

Corresponding author: Talalayeva Olga Sergeyevna, Candidate of Medical Sciences, Associate Professor of the Department of pathological physiology of ASMU, Barnaul.

656038, Barnaul, Lenina Prospekt, 40,

Tel: 8 (3852) 24-18-56.

E-mail: talalaeva_olga@mail.ru

UDC 616.322:616.36-009

POTENTIAL FOR USE OF FOMES OFFICINALIS EXTRACT IN THERAPY OF TOXIC HEPATITIS

Altai State Medical University, Barnaul
Center of medico-biological research, biomedical laboratory, Barnaul

O.N. Mazko, O.G. Makarova, L.G. Dvornikova, A.P. Pashkov

As a result of the research, it was established that the Fomes officinalis extract has a hepatoprotective effect (in a dosage of 80 mg / kg rat weight), one of the mechanisms of action of which is antioxidant activity. The introduction of the studied extract also contributes to the normalization of lipidic and carbohydrate metabolism. The received data testify to expediency of development on the basis of a polypore extract, a medication for treatment of toxic diseases of a liver.

***Key words**: extract, Fomes Officinalis, hepatoprotection, toxic hepatitis.*

Introduction

Pharmacotherapy of diseases of the organs of the hepatobiliary system uses various groups of drugs ("Heptral", "Legalon", etc.), but a special place among them is occupied by medicines, different in structure and mechanism of action, but having selective action against the liver - hepatoprotectors. The action of the latter is aimed at normalization of metabolic processes and homeostasis in the liver, increasing the resistance of hepatocytes to pathogenic influences, stimulation of regenerative processes, restoration of hepatic parenchyma and recovery of its physiological functions [1]. Despite the fundamental discoveries of recent years in the field of hepatology, many aspects of treatment and prevention of liver disease remain unexplored. Therefore, today it has become urgent to develop new phytogenic drugs for treatment and prevention of liver diseases on the basis of raw materials growing on the territory of Russia.

The research objective was to study the effect of the Fomes Officinalis extract on the course of toxic hepatitis in rats.

Materials and methods

The object of the research was the extract of the fruit body of the Fomes Officinalis, which was a brown color substance, having a specific odor and a bitter taste, soluble in alcohol by 40% ethanol.

In vivo experiments were carried out in winter-spring period on certified animals of both sexes (Wistar rats), provided by the Institute of Cytology and Genetics of the SB RAS. Animals were in standard conditions of detention under natural light conditions, free access to water and food. The experiments were carried out in accordance with the "Rules of Laboratory Practice in the Russian Federation" (Order of the Ministry of Health of the Russian Federation No. 708n of August 23, 2010).

The study of hepatoprotective activity was carried out on rats weighing 210-265 grams. Experimental toxic hepatitis was caused by intra-gastric administration to animals of 1 ml of a 25% oily solution of carbon tetrachloride for 6 days. The extract of the fruit body of Fomes Officinalis (at a dosage of 80 mg / kg) and the reference preparation Legalon (200 mg / kg) were orally administered as a suspension in a 2% starch paste once a day for 21 days after the formation of the model pathology. On the 28th day, under ether anesthesia, euthanasia of experimental animals was carried out by decapitation, followed by the collection of biomaterials (blood, liver) for research [2,3].

Screening tests were used for the initial assessment of hepatoprotective activity: the survival rate of animals, the change in body weight and the weight of the liver were determined. In order to gain in-depth knowledge about the effect of experimental drugs on the course of hepatitis, biochemical values of rat blood characterizing liver function were evaluated [3]. Levels of glucose, albumin, total bilirubin, total cholesterol, triglycerides and urea in the blood serum of animals were determined photometrically with the help of using reagent kits of the company Vector-Best (Novosibirsk). Activity of enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT) and alkaline phosphatase (AFP) was determined by photometric kinetic method using the sets of the firm "Human, GmbH" (Germany).

The software package Statistica 6.1 was used for statistical processing of the results. The data are presented as the mean (M) and the standard error of the mean value (m). The correspondence of the samples to the law of normal distribution was estimated by the Shapiro-Wilk criterion. To test statistical hypotheses about the difference between the groups studied, the Mann-Whitney, Student, and Wilcoxon criteria were used. The investigated parameters of the control group were evaluated

in comparison with the indices of intact animals, the indices of other groups were compared with the control animals.

Results and discussion.

Intoxication with carbon tetrachloride was accompanied in animals of the control group by a decrease in survival rate to 73.3%, a decrease in body weight by 15%, and an increase in the mass of the liver by 19.4% compared to the intact group. In addition, hyperenzymemia was observed, indicating the destruction of hepatocytes and progression of cholestasis, which led to an increase in the serum of control animals of cholesterol, triglycerides, urea and bilirubin (up to 1.74 ± 0.19, 1.73 ± 0.25, 5, 15 ± 0,40 and 6,25 ± 0,90 mmol/L, as relevant) and a decrease in the glucose concentration and albumin in comparison with the intact group by 35% and 32%, as relevant (Table 1).

Table 1

Influence of the extract of the fruit body of the Fomes Officinalis and the medication "Legalon" on the course of experimental toxic hepatitis

Index	Study group			
	Intact	Control	Extract 80 mg/kg	Legalon 200 mg/kg
Screening tests				
Survivability,%	100.0	73.3	100.0	100.0
Mass of the liver, mg/g	33,5±0,50	40,0±1,05	36,5±1,09	33,7±1,16
Rate of change in body weight, g.	+15.0	-30.0	+7.9	+30.0
Biochemical values				
Alanine transaminase, U/l	58,6±3,9	110,4±8,1*	69,2±5,6*	74,8±6,3*
Aspartate transaminase, U/l	196,6±6,0	312,6±27,9*	285,5±24,3	303,3±19,0
Alkaline phosphatase, U/l	374,0±18,4	645,6±45,8*	426,7±41,3*	469,4±53,4*
Gamma-glutamyltransferase U/l	2,28±0,67	3,36±1,48*	2,25±0,98*	2,02±0,46
Glucose, mmol/L	7,70±0,45	5,00±0,28*	7,80±0,91*	5,36±0,23
Albumin, г/л	69,5±0,7	47,1±3,6*	50,6±9,7	37,1±2,9*
Bilirubin, umol/l	4,03±0,66	6,25±0,90	3,10±0,32*	5,90±0,48
Cholesterol, mmol/L	1,42±0,10	1,74±0,19	1,41±0,15*	1,13±0,13
Triglyceride, mmol/L	1,60±0,06	1,73±0,25	1,39±0,12*	1,60±0,27
Urea, mmol/L	4,66±0,34	5,15±0,40	4,65±0,32*	3,96±0,12*

*Note: *the differences are statistically significant at p <0.05*

The data of Table 1 indicate that the Fomes Officinalis extract and the reference medicatopn Legalon reduced the hepatotoxic effect of carbon tetrachloride: the survival rate of rats increased to 100%; the body weight of animals increased by 7.9 and 30.0 g, as relevant; the mass of the liver decreased by an average of 12%. A statistically significant decrease in the activity of ALT, GGT, and ALP was observed in the group of animals that received Fomes Officinalis extract at a dosage of 80 mg / kg compared to the control group (by 37%, 33% and 34%, respectively). The activity of AST in the blood serum of animals that received the polypore extract at this dosage, as compared to this indicator of control animals, decreased, but no statistically significant differences were revealed. The introduction of the extract studied led to a reduction in the levels of cholesterol, triglycerides and urea in the blood plasma compared to the control group by 19%, 20% and 10%, respectively. The restoration of albumin levels in the blood was not observed with the use of the extract and in the group of animals taking the reference medication. As can be seen in the experimental data, intoxication with carbon tetrachloride results in an increase in the level of bilirubin and cholesterol by 1.5 and 1.2 times, as relevant, whereas the administration of the fomes officinalis extract and Legalon solution contribute to a significant decrease in these indicators in blood serum.

Conclusion

The final result of the experiment revealed that the Fomes Officinalis extract shows hepatoprotective properties in experimental toxic hepatitis caused by carbon tetrachloride.

References

1. Agarkova Ye.V. The use of Essliver forte in the complex therapy of diseases of the hepatobiliary zone. *RMJ. Diseases of the digestive system.* 2008; 2: 68-70.

2. Dvornikova L.G., Turetskova V.F., Zamyatin S.V., Mazko O.N., Zolovkina A.G. et al. *Izvestia of Samara Scientific Center of the Russian Academy of Sciences.* 2012; 5(3): 711-714.

3. Khabriyev R.U. ed., *Manual on experimental (preclinical) study of new pharmacological substances.* 2nd ed. Moscow: Meditsna; 2005.

Contacts

Corresponding author: Mazko Olesya Nikolayevna, Candidate of Biological Sciences, Senior Scientific Researcher of the Center of medico-biological research, Barnaul.

656038, Barnaul, ul. Nekrasova, 65.

Tel.: (3852) 669927.

E-mail: olesia.mazko@yandex.ru

UDC 615.322(571.15)

STUDY OF THE POLYSACCHARID COMPLEX LAVATERA THURINGIACA L., GROWING IN ALTAI KRAI

Altai State Medical University, Barnaul

L.M. Fedoseyeva, O.A.Myznikova

The aim of the work is to study the qualitative composition of polysaccharides and determine the quantitative content of the sum of polysaccharides in the Lavatera thuringiaca herb growing on the territory of Altai Krai. By means of qualitative reactions, the presence of mono- and polysaccharides in the raw material was confirmed. Monosaccharides were identified by thin layer chromatography: arabinose, glucose, galactose. By the method of gravimetry, the content of the sum of polysaccharides in the Lavatera thuringiaca herb gathered in different regions of Altai Krai were established: 6,85-8,39%.

***Key words**: Lavatera thuringiaca L., polysaccharide complex.*

Introduction

Plants of the Malvaceae family (Malvaceae Juss) are used in folk medicine as an expectorant, anti-inflammatory, analgesic, coating agent. In scientific medicine only althaea officinalis (Althaea officinalis L.) and Althae armenian (Althaea armeniaca Ten.) are used, the herb and roots of which contain polysaccharide complexes, which allows using them for the treatment of colds, acute respiratory and some other diseases [1].

On the territory of Altai Krai the range of distribution of different kinds of althea is very limited: they grow only in wildlife preserves. Consequently, there is a need to search for additional sources of raw materials among closely related species.

One of such plants is the Khatma Thuringian (Lavatera thuringiaca L.). The advantages of this type are: the structural diversity of a complex of biologically active compounds (vitamins, polysaccharides, phenolic compounds); wide distribution area on the territory of Altai Krai (meets everywhere) [2-3].

The research objective is to research the qualitative composition of sugars and determine the quantitative content of the sum of polysaccharides in Lavatera thuringiaca L., which grows on the territory of Altai.

Materials and methods

The object of the study was the use of the Lavatera thuringiaca L. harvested in the territory of Biysk, Bystroistoksky, Kalmansky, Krasnogorsky, Pervomaysky, Soltonsky and Tselinny districts of Altai Krai. Harvesting of grass was conducted in 2017 in different phases of vegetation: budding (early June), flowering (mid-July), and fructification (end of August).

Water extracts from Lavatera thuringiaca L. were prepared in the ratio "raw-extractant" - 1:10. To confirm the presence of sugars in the extracts, qualitative reactions were carried out: reaction with Fehling's reagent, reaction of a silver mirror, Molisch reaction. The presence of polysaccharides was confirmed by precipitation with ethyl alcohol of 96%. Starch was identified by reaction with a solution of Lugol; mucus - by reaction with an alkali solution [4-5].

The composition of raw monosaccharides was studied by thin layer chromatography (TLC). Hydrolysis of the polysaccharides with hydrochloric acid concentrated in a boiling water bath for 30 minutes was carried out. 40% solution of sodium hydroxide was added dropwise to the hydrolysates to get a solution with a pH of 4.0-4.5 [6]. The solutions under investigation and solutions of standard samples (CO) of monosaccharides: arabinose, galactose, glucose, xylose, fructose were applied on the start line of the chromatographic plates "Sorbfil". Standard samples were purchased from CJSC "VECTON".

A 4:1:5 alcohol-n-butyl-acetic acid-ice-water (IV) solvent system was used as the mobile phase [7]. Chromatograms were displayed by successive treatment of sodium carbonate with a solution of 20% and picric acid with a solution of 1%. The plates were heated in a drying cabinet. The color of the spots was marked. Rf values were calculated.

The quantitative determination of the sum of polysaccharides in Lavatera thuringiaca L., harvested at different stages of vegetation and from different growth sites, was carried out by gravimetry according to the State Pharmacopoeia (GF) of the XIIIth publication [6]. The data obtained were statistically processed in accordance with the requirements of the GF XIII edition (FS.1.1.0013.15) [6].

Results and discussion

With aqueous extracts of Lavatera thuringiaca L., qualitative reactions to sugars were carried out. Positive results of the reactions indicate the presence of free and bound sugars in the raw material under study (Table 1).

Table 1

Results of qualitative reactions to monosaccharides and polysaccharides

Name of reagents (reactions)	Results of reactions	The presence of ALS
Feling's Reagent	The precipitation of a brick-red precipitate	Reducing sugars are present
Silver nitrate ammonia solution 2.5% (silver mirror reaction)	The precipitation of brown precipitate	
α-naphthol alcohol solution 20%, concentrated sulfuric acid (Molish reaction)	Formation of a red ring at the interface of two phases	Monosaccharides, disaccharides, polysaccharides are present
Ethyl alcohol 96% (polysaccharide precipitation reaction)	Precipitation of gelatinous sediment	polysaccharides are present
Sodium hydroxide solution of 30%	Bright yellow color	mucus are present
Lugolya solution	Brown staining	Starch is missing

It is established that the structure of the polysaccharide complex Lavatera thuringiaca L. includes mucus. Starch in the investigated raw material was not detected.

Further, acid hydrolysis of polysaccharides was carried out and the monosaccharide composition of the raw material was studied by the TLC method in a 4: 1: 5 BUV system. After chromatography, the plates were air dried; the sodium carbonate was treated with a solution of 20% and picric acid with a solution of 1%; then they were heated in an oven. There was observed the appearance of orange spots on a yellow background (Figure 1). The Rf values of the test and standard samples were calculated (Table 2).

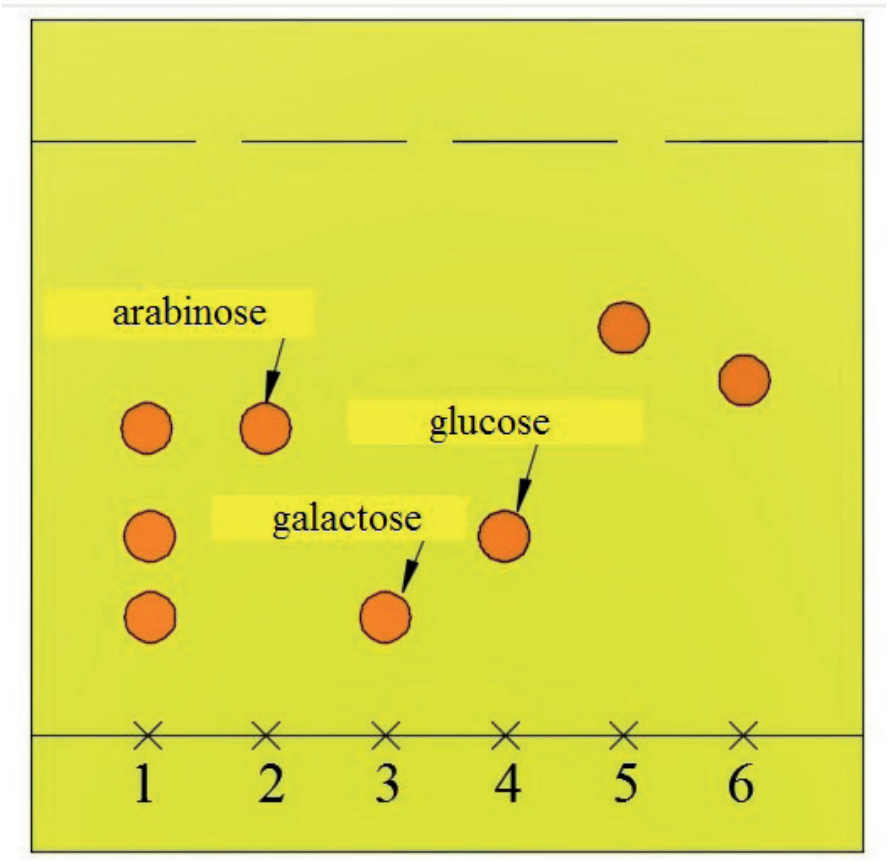

Figure 1.
Chromatogram of sugars Lavatera thuringiaca L. in the system BUV (4: 1: 5): 1 - aqueous extract of Lavatera thuringiaca L., 2-6 - CO monosaccharides

Table 2

The results of the determination of the monosaccharide composition of Lavatera thuringiaca L. by the TLC method in the BWA system (4: 1: 5)

№ CO	**Name of** CO	**Coefficient Rf** CO	Coefficient Rf monosaccharides of raw materials
2	L(+)-Arabinose	0,43±0,03	0,43±0,04
3	D(+)-Galactose	0,34±0,03	0,34±0,03
4	D(+)-Glucose	0,37±0,04	0,37±0,03
5	D(+)-Xylose	0,49±0,05	-
6	D(-)-Fructose	0,45±0,04	-

Table 2 shows that Lavatera thuringiaca L. monosaccharides are represented by arabinose (Rf = 0.43 ± 0.04), galactose (Rf = 0.34 ± 0.03), glucose (Rf = 0.37 ± 0.03.

Further, there was carried out quantitative determination of the sum of Lavatera thuringiaca L. polysaccharides by using gravimetry method according to the GF XIII method [6]. The results of the study are presented in Table 3.

Table3

Results of quantitative determination of the sum of polysaccharides in Lavatera thuringiaca L., harvested in different phases of vegetation and in different regions of growth

Phenological phase	Metrological characteristics, **P=95%, n=5, f=2,78**		
	$\bar{x}\pm\Delta\bar{x}$, %	s	$\bar{\varepsilon}$, %
bud-formation phase	7,63±0,15	0,09	1,18
flowering phase	7,60±0,18	0,10	1,35
fruiting stage	6,82±0,16	0,09	1,33
Area of harvesting	Metrological characteristics, **P=95%, n=5, f=2,78**		
	$\bar{x}\pm\Delta\bar{x}$, %	s	$\bar{\varepsilon}$, %
Biysky	7,60±0,18	0,10	1,35
Bystroistoksky	7,78±0,15	0,09	1,14
Kalmansky	8,39±0,16	0,10	1,08
Krasnogorsky	7,67±0,19	0,11	1,48
Pervomaysky	8,18±0,24	0,14	1,69
Soltonsky	7,28±0,18	0,10	1,42
Tselinny	6,85±0,17	0,10	1,42

It is established that the greatest accumulation of the sum of polysaccharides in Lavatera thuringiaca L. occurs in the budding phase (June) and flowering (July). Therefore, this period is optimal for raw material procurement.

Next, we compared the quantitative content of the sum of polysaccharides in grass harvested in different regions of Altai Krai, differing in climatic and ecological conditions (Table 3). It was found out that the content of the sum of polysaccharides varies insignificantly within the range of 6.85-8.39% (Figure 2).

The results of the study indicate the possibility of harvesting Lavatera thuringiaca L. throughout the territory of Altai Krai.

Conclusion

1. During the work it was established that Lavatera thuringiaca L. contains carbohydrates of different structure: monosaccharides and polysaccharides. Monosaccharides are represented by arabinose, glucose, and galactose. The composition of the polysaccharide complex includes mucus.

2. The maximum accumulation of polysaccharides in Lavatera thuringiaca L. occurs in the budding and flowering phases.

3. The content of the sum of polysaccharides in Lavatera thuringiaca L., harvested in different regions of Altai Krai, is 6.85-8.3%.

The results of the research allow us to consider Lavatera thuringiaca L. as an alternative source of medicinal plant material.

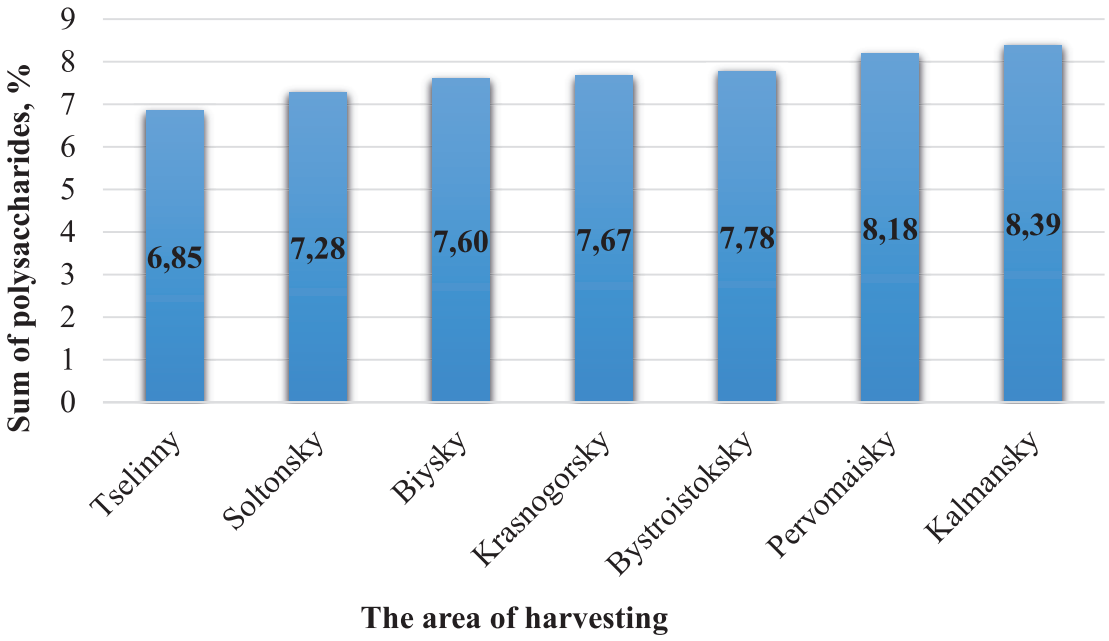

Figure 2.
The content of the sum of polysaccharides in Lavatera thuringiaca L., depending on the area of the harvesting

References

1. Drozdova I.L. Pharmacognostic study of some representatives of the Malvaceae family: Dis. ... cand. farm. sciences. Kursk, 2000.

2. Fedoseyeva L.M., Myznikova O.A., Kudrikova L.E. Study of phenolic compounds of the aerial part of Lavatera thuringiaca L, growing on the territory of Altai Krai. *Chemistry of plant raw materials*. 2017; 2: 107-112.

3. Fedoseyeva L.M., Myznikova O.A. Determination of the composition of ALS Lavatera thuringiaca L., which grows on the territory of Altai Krai. *Topical problems of pharmacology and pharmacy*. 2016; 13: 191-197.

4. Bryukhanov V.M., Fedoseyeva L.M. *Leather bergenia*. Barnaul, 2006.

5. Konopleva M.M. Pharmacognosy: natural biologically active substances. Vitebsk, 2010.

6. State Pharmacopoeia of the Russian Federation [Electronic resource]: Ministry of Health of the Russian Federation. M .: 2015. Available at: http://www.femb.ru/feml.

7. Belikov V.G., Menkov S.V., Ligay L.V. *A study of the dynamics of accumulation of polysaccharides in Lavatera thuringiaca L. Development, research and marketing of new pharmaceutical products: a collection of scientific papers*. Pyatigorsk, 2005; 60: 185-187.

Contacts

Corresponding author: Myznikova Olga Aleksandrovna, postgraduate student of the Department of Pharmacy of ASMU, Barnaul.
656038, Barnaul, Lenina Prospekt, 40.
Tel. (3852) 24-02-78.
E-mail: olga_myznikova@mail.ru

UDC 612.1:615.324-092.4

ADAPTOGENIC EFFECT OF PRODUCTS OF ANTLER STAG BREEDING ON THE STATE OF HEMOSTATIC SYSTEM IN RATS BY SUPRATHRESHOLD PHYSICAL LOAD

Altai State Medical University, Barnaul

A.A. Blazhko, I.I. Shakhmatov, V.I. Kiselev, A.Yu. Zharikov

The article presents the results of the study of the hemostatic system of rats by means of an integral method – thromboelastography by suprathreshold physical load without prior intake of the adaptogene and by preliminary course 30-day intake of the concentrate containing blood and histolysate of the Siberian stag reproductive organs. There was also studied the influence of the course intake of the concentrate and separate additives (glucose, ascorbic acid, fruit essence) contained in the studied concentrate on the hemostatic system parameters. It was shown, that the preliminary course 30-day intake of the concentrate decreases the risk of development of the state of thrombotic readiness in rats after 8-hour physical load due to the increased intake of the concentrate of blood plasm fibrinolytic activity. It was also proved, that the components increasing adaptive qualities of the hemostatic system in rats are active agents contained in blood and histolysate of the Siberian stag reproductive organs.

Key words: *thromboelastography, histolysate of the Siberian stag reproductive organs, hemostatic system.*

Introduction

Physical load is one of the most common types of stress in the human body which by regular activity can increase its resistance through mechanisms of cross adaptation [1, 2]. At the same time, the suprathreshold physical load can lead to the development of damage to organs and systems, causing a state of distress in the body [3]. It is known that distress from the hemostasis system is manifested by the development of the state of thrombotic readiness [3, 4]. There are many methods for recognizing the development of thrombotic readiness, but the overall picture of coagulation from the onset of coagulation to the fibrinolysis process with the definition of viscoelastic properties of the clot can be fixed by only an integral method - thromboelastography [5, 6].

Earlier in our laboratory, it was established that in order to avoid the development of thrombotic readiness state in case of suprathreshold stress, it is necessary to increase the resistance of the body and the hemostasis system in particular, by physical training or by taking plant adaptogens [7, 8]. Adaptogenes of animal origin include products of antler stag breeding, which increase the mental and physical performance of the organism [9]. It is known that in products of antler stag breeding there are no prohibited doping substances or similar analogs [10]. Moreover, it was noted that concentrates a histolysate from male genital organs containing in addition to stag blood have a more pronounced tonic effect due to an increase in biosynthetic activity in rat skeletal muscle cells [11].

Earlier, we already considered the effect of the exchange of products of antler stag breeding on the state of the hemostasis system [12, 13]. However, the effect of the preliminary intake of the concentrate and its constituents on the indices of the hemostatic system, estimated by the method of thromboelastography, was not studied by suprathreshold physical load.

The research objective is to evaluate the reactions of the hemostasis system by the method of thromboelastography in rats after suprathreshold load by a preliminary course intake of concentrate containing blood and histolysate from the reproductive organs of stag and also to exclude the possible adaptive effect on the hemostatic system of supplements (glucose, ascorbic acid, fruit essence) included in the composition of the concentrate used.

Materials and methods

The studies were performed in 50 male Wistar rats weighing 250 ± 30 g. The animals were divided into five groups (intact and four experimental groups).

The first two experimental groups of animals were subjected to an 8-hour physical load in the form of an imposed run in a treadmill with a rotation speed of 6-8 m/min. In this case, the animals of the second experimental group, in contrast to the first, previously received the concentrate for 30 days. Immediately after the end of the physical load, the blood from the hepatic sinus was taken for the study in a volume of 5 ml into a polystyrene syringe containing 3.8% sodium citrate solution (blood and citrate ratio 9:1) under anesthesia.

The third and fourth groups of experimental animals took a concentrate (the third group) and, separately, additives (glucose, ascorbic acid, fruit essence), contained in the concentrate used (the fourth group) for 30 days, and were not subjected to physical load. Blood was taken on the 31st day of the intake of concentrate and supplements.

Experimental animals took a concentrate containing blood and histolysate of the genital organs of the stag males produced under the trade name "Pantohematogen (Lubyangem)" (FSBRI "All-Russian Scientific Research Institute of Antler Stag Breeging" of FASO of Russia, Barnaul), 4.5 ml per day. Calculation of the dose of concentrate for rats was performed taking into account the coefficients of interspecific counting [14]. Experimental animals were kept in individual cells and an aqueous solution of the concentrate was taken orally from individual drinkers. The solution was prepared by adding 4.5 ml of concentrate to water, bringing the solution to a total volume of 40 ml (the daily rate of water intake for these rats detected by us prior to the beginning of the experiment).

The animals of the fourth experimental group took supplements (glucose, ascorbic acid, fruit essence) contained in the concentrate under study, in a volume of 1.7 ml per day for 30 days. The volume of 1.7 ml of the concentrate additives was diluted with water to 4.5 ml; thus, the putative active substance (blood and histolysate of the genital organs of the stag males) was replaced by water in appropriate proportions. Intact animals took water in the same volume as the experimental animals.

The thromboelastogram was recorded on a RotemGamma apparatus (Germany) in Natem mode for 35 minutes using the Star-tem activator. The following parameters of thromboelastogram were assessed:

- Coagulation time (CT) - the time from the moment of application of the activator to reaching a 2 mm amplitude by the thromboelastogram, this interval reflects the initiation phase of blood coagulation [15].

- Clot formation time (CFT) - time of amplitude change of thromboelastogram from 2 mm to 20 mm, this index characterizes the phase of strengthening of the process of thrombus formation [15].

Angle alpha (ALP) - the angle formed by the longitudinal axis of the thromboelastogram and the straight line drawn tangentially to the thromboelastogram from the point corresponding to the clot amplitude of 2 mm. This value reflects the kinetics of clot formation and characterizes the propagation phase [16].

- Maximum clot firmness (MCF) - an index corresponding to the maximum amplitude of the clot and reflecting the function of platelets and fibrinogen [8].

- Maximum lysis (ML) - an indicator characterizing the level of maximum fibrinolysis recorded during the analysis. It is defined as finding the lowest amplitude after reaching the MCF.

The use of rats in experiments was carried out in accordance with the European Convention for the Protection of Vertebrate Animals used in the experiment, as well as the Directives - 86/609 /EEC. Anesthesia of animals was carried out in accordance with the "Rules of work with the use of experimental animals."

The data obtained during the study are presented in tables in the form (m [25-75%]), where m is the median in the sample multitude; [25-75%] - the 25th and 75th percentiles. The statistical analysis was carried out on a personal computer using the Statistica 6.0 application package (StatSoft, USA). The significance of the differences was estimated using the nonparametric Mann-Whitney U-test, since the features examined did not obey the normal distribution. Differences were considered significant at a level of statistical significance $p < 0.05$.

Results and discussion

As follows from the data presented in Table 1, an 8-hour physical load induced a shortening of coagulation time (CT) by 26% ($p1 = 0.002$) in rats (the first experimental group), an increase in the "alpha-alpha" index by 10% ($p1 < 0.001$) and a reduction in clot formation time (CFT) by 22% ($p1 = 0.028$) compared to that of intact animals, suggesting a shift in the hemostatic system towards hypercoagulability and consistent with the already described data obtained by coagulation tests. The maximum clot firmness (MCF) decreased by 11% ($p1 = 0.018$) at the end of the 8-hour physical load as compared to the intact animals, which indicated a decrease in the platelet count and a decrease in the fibrinogen level in the blood of experimental animals due to their consumption for intravascular thrombus formation. The maximum lysis of the clot (ML) after an 8-hour load, unlike intact animals, was not determined, which indicated a decrease in the fibrinolytic activity of the blood plasma. Thus, changes in the described indices of thromboelastogram indicate that an 8-hour physical load induces a state of thrombotic readiness in rats [3, 4].

In animals of the second experimental group which were exposed to 8-hour physical load after a 30-day course of the intake of concentrate containing blood and histolysate from the reproductive organs of the stag, the thromboelastogram indices did not differ from those in intact animals. Consequently, the preliminary administration of the adaptogen reduced the risk of thrombotic readiness in rats after an 8-hour physical load.

As follows from the data presented in Table 2, in rats, a 30-day intake of a concentrate containing blood and histolysate from stag reproductive organs caused a decrease in the maximum clot firmness by 12% ($p1 = 0.014$) and an increase in the maximum lysis (ML) by 125% ($p1 = 0.021$) in comparison with intact animals, which indicates an increase in the fibrinolytic activity of blood plasma. This adaptive effect of concentrate intake may be associated with activation of endothelial release of the tissue plasminogen activator (t-PA) and a de-

crease in the amount of plasminogen activation inhibitor (PAI-1) [17].

Admission of additives (glucose, ascorbic acid, fruit essence) contained in the test concentrate for 30 days in rats did not cause changes in the thromboelastogram score in comparison with intact animals. Therefore, it can be assumed that active components contained in the blood and histolysate from the reproductive organs of stags are the active components of the concentrate that increase the adaptability of the hemostasis system, manifested in the increase in the fibrinolytic activity of the blood plasma.

Conclusions

1. The ultra-threshold 8-hour physical load causes the development of thrombotic state in rats.

2. A preliminary 30-day intake of a concentrate containing blood and histolysate from the reproductive organs of stags reduces the risk of the development of thrombotic readiness in rats after a supra-threshold exercise.

3. Increased adaptability of the hemostasis system when receiving a concentrate containing blood and histolysate from the reproductive organs of stags consists in the increase the fibrinolytic activity of the blood plasma.

4. The active ingredients contained of the used Pantohematogen (Lubyangem) concentrate which enhance the adaptive properties of the hemostasis system in rats are the active substances contained in the blood and histolysate from the reproductive organs of stag males.

Table 1.

Indices of thromboelastogram: intact animals; experimental rats after an 8-hour physical load; experimental animals after 8-hour physical load with a preliminary 30-day intake of concentrate

Indices	Intact rats (n=10)	8-hour physical load (n=10)	8-hour physical load on the 31st day of concentrate intake(n=10)
Coagulation time (CT), sec	193,0 [167,3-206,3]	143,0 [114,0-163,0] p1=0,002 (Δ1 – 26 %)	176,0 [163,5-183,5] p1=0,349
Clot formation time (CFT), sec	76,0 [64,3-106,5]	59,5 [52,5-67,8] p1=0,028 (Δ1 – 22 %)	74,0 [70,0-80,0] p1=0,690
Angle of alpha (ALP), 0	73,0 [68,5-77,0]	80,0 [79,5-82,0] p1<0,001 (Δ1 + 10 %)	74,5 [68,0-77,0] p1=0,939
Maximum clot firmness (MCF), mm	68,0 [62,0-70,8]	60,5 [59,0-65,3] p1=0,018 (Δ1 - 11 %)	67,5 [66,8-70,3] p1=0,562
Maximum lysis (ML), %	4,0 [1,0-6,0]	0,0 [0,0-1,0] p1=0,013 (Δ1 - 100 %)	3,0 [0,8-6,5] p1=0,894

Note: the results are presented in tables in the form (m [25-75%]), where m is the median in the sample multitude; [25-75%] - the 25th and 75th percentiles. Δ1 - statistically significant difference in the experimental group with intact animals at $p < 0.05$; p1 - the level of significance of differences in the experimental group with intact animals. CT - the coagulation time, CFT - the clot formation time, MCF - the maximum clot firmness, ML - the maximum lysis.

References

1. Agadzhanyan N.A., Bayevsky R.M., Beresneva A.P. *Theory of health and adaptation problems*. Stavropol: SGU, 2000.

2. Pshennikova M.G. The phenomenon of stress. Emotional stress and its role in pathology. *Journal of Pathophysiology and Experimental Therapy.* 2001; 3: 28 – 40.

3. Shakhmatov I.I. Single physical exercises and immobilization of different duration impact haemostatic system reactions. *Fundamental research*. 2010; 3: 144-150.

4. Momot A.P. et al. *Modern methods of thrombotic readiness detection.* Barnaul, 2011.

5. Trzebicki J., Kuzminska G., Domagala P. Thromboelastometry – a new method supporting the therapeutical decisions in the coagulopathy based on the Hartet's thromboelastography. *Pol. Merkur. Lekarski.* 2009; 27(158): 85-91.

6. Theusinger O.M., Wanner G.A., Emmert M.Y., Billeter A., Eismon J., Seifert B., Simmen H.P., Spahn D.R., Baulig W. Hyperfibrinolysis diagnosed by rotational thromboelastometry (ROTEM1) is associated with higher mortality in patients with severe trauma. *Anesth Analg*. 2011; 113(5): 1003-1012.

7. Alekseeva O.V., Bondarchuk Yu.A., Shakhmatov I.I., Vdovin V.M., Bondarenko N.A. Adaptive changes in the parameters of peripheral blood

Table 2.

Indices of thromboelastogram: intact animals; experimental group of rats taking concentrate for 30 days; experimental animals taking the concentrate additives for 30 days

Indices	Intact rats (n=10)	30-day intake	
		concentrate (n=10)	concentrate additives (n=10)
Coagulation time (CT), sec	193,0 [167,3-206,3]	209,0 [173,0-250,0] p1=0,270	189,5 [181,5-197,8] p1=0,979
Clot formation time (CFT), sec	76,0 [64,3-106,5]	101,0 [90,0-106,0] p1=0,064	81,0 [72,8-90,0] p1=0,655
Angle of alpha (ALP), 0	73,0 [68,5-77,0]	73,0 [71,0-76,0] p1=0,944	71,5 [70,3-74,8] p1=0,930
Maximum clot firmness (MCF), mm	68,0 [62,0-70,8]	60,0 [57,0-65,0] p1=0,014 (Δ1 – 12 %)	67,0 [63,3-72,0] p1=0,396
Maximum lysis (ML), %	4,0 [1,0-6,0]	9,0 [5,0-12,0] p1=0,021 (Δ1 + 125 %)	5,5 [2,0-10,0] p1=0,482

Note: the results are presented in tables in the form (m [25-75%]), where m is the median in the sample multitude; [25-75%] - the 25th and 75th percentiles. Δ1 - statistically significant difference in the experimental group with intact animals at $p < 0.05$; p1 - the level of significance of differences in the experimental group with intact animals. CT - the coagulation time, CFT - the clot formation time, MCF - the maximum clot firmness, ML - the maximum lysis.

during physical training. *Bulletin of Siberian Medicine*. 2005; 4: 35.

8. Shakhmatov I.I., Bondarchuk Yu.A., Vdovin V.M., Alekseyeva O.V., Kiselev V..I Hemostasis disorders and their correction by adaptogen. *Journal of Pathophysiology and Experimental Therapy*. 2010; 2: 43-46.

9. Suslov N.I., Guryanov Yu.G. *Products based on pantogematogen. Mechanisms of action and application features.* Novosibirsk: Siberian University Publishing House, 2008.

10. Semenov V.A., Latkov N.Yu., Koshelev Yu.A., Poznyakovsky V.M. Application of pantogematogen in sports medical practice. *Food Processing: Techniques and Technology.* 2014; 2: 113-117.

11. Zharikov A.Yu., Lunitsyn V.G., Lampatov V.V., Motin Yu.G., Talalayeva O.S., Eliseyev D.V., Pavlyashik G.V. Influence of new agents from raw materials of fawn`s antlers on biosynthetic processes in rats skeletal muscles cells in conditions of long physical activity. *Bimedicine.* 2016; 1: 90-94.

12. Bondarchuk Yu.A., Blazhko A.A., Alekseyeva O.V., Shakhmatov I.I., Nikolaev V.Yu. Effect of the course of reception of Eleutherococcus and Pantogematogen on the state of the hemostasis system. *Modern problems of science and education.* 2016; 6. Available at: https://www.science-education.ru/en/article/view?id=25928 Accessed on 11/25/2017.

13. Blazhko A.A., Shakhmatov I.I., Moskalenko S.V., Lycheva N.A. Activating effect of pantogematogen on the reaction of the hemostasis system. *Siberian Scientific Medical Journal*. 2016; 36(4): 51-55.

14. Khabriyev R.U. *Manual on experimental (preclinical) study of new pharmacological substances*. Moscow: Meditsina, 2005.

15. Kawassaki J., Katori N., Kodaka M., Miyao H., Tanaka K.A. Electron microscopic evaluation of clot morphology during thrombelastography. *Anesth. Analg*. 2004; 99(5): 1440-4.

16. Johansson P.I., Svenders M.S., Salado J., Bochsen L., Kristensen A.T. Investigation of the thrombin-generating capacity, evaluated by thrombogram, and clot formation evaluated by thrombelastography of platelets stored in the blood bank for up to 7 days. *Vox Sang*. 2008; 94(2): 113-8.

17. Melnikova Yu.S., Makarova T.P. Endothelial dysfunction as the central link of the pathogenesis of chronic diseases. *Kazan Medical Journal*. 2015; 96 (4): 659-665.

Contacts

Corresponding author: Blazhko Aleksandr Aleksandrovich – Lecturer of the Department of normal physiology of ASMU, Barnaul.
656038, Barnaul, Lenina Prospekt, 40.
Tel.: 8-903-073-6667.
Email: blazhko_1990@mail.ru

UDC 616-022:616.983-08

SUSTAINABLE THERAPY OF UROGENITAL CHLAMYDIOSIS

Irkutsk State Medical University, Irkutsk
Regional dermatovenerologic dispensary, Irkutsk

I.O. Malova, I.A. Sidorova

There is presented the comparative characteristic of 2 methods of treatment of urogenital chlamydiosis in women of reproductive age: doxycycline monohydrate monotherapy and combination of doxycycline monohydrate with interferon inducer Meglumini acridonacetas. In 2 groups of comparison there was performed the analysis of indicators of neutrophils functional activity (phagocytic index, phagocytic number, NBT-test – spontaneous and induced) and also the rate of T-lymphocytes producing gamma interferon in the total pool of T-lymphocytes in peripheral blood and concentration of gamma interferon in the blood serum during treatment.
Key words: *urogenital chlamydiosis, neutrophils functional activity, T-lymphocytes, gamma interferon, doxycycline, Meglumini acridonacetas.*

Urogenital chlamydial infection is one of the most spread sexually transmitted infections [20, 21]. According the World Health Organization (WHO), in the world there are annually registered about 100 mln new cases [23, 24].

Chlamydial infection (CI) is widely spread among persons of reproductive age, while young age in women is one of the basic factors of infection risk. Due to cylindrical epithelium tropism, *C. trachomatis effects urogenital organs and presents one of the important reasons of reproductive function disorder.*

Inflammation by urogenital chlamydiosis (UGC) often lacks symptoms. The absence of timely diagnosis and, consequently, therapy leads to the development of complications in women – such as inflammatory diseases of pelvic organs (POID), extra-uterine pregnancy and tubal infertility. A special problem are the cases of CI persistence, when POID proceed without symptoms of inflammation [9, 23].

Basic factors of non-specific antimicrobial protection of organism are phagocytosis and complement system. Chlamydia can persist in phagocytes (neutrophils and macrophages). In patients with chronic CI there was registered the reduction of digestive function of peripheral blood neutrophils [2].

The influence of chlamydia suppresses not only phagocytic system, but also T-cell segment. Patients with urogenital chlamydiosis showed the decrease of total T-lymphocytes (CD3+) in peripheral blood with the disorder of their functional activity [7].

The activator of macrophages is γ-interferon (γ-IF), which is mainly produced by T-lymphocytes. Low concentration of γ-IF is the main condition for persistence development [4]. The factors of persistent chlamydial infection are also sub-therapeutic doses of antibiotics and treatment with drugs of low chlamydia activity [17].

It is known, that high doses of γ-IF inhibit growth of chlamydia. Low doses, on the contrary, induce the development of morphologically aberrant forms of inclusions [8].

Sufficient production of γ-IF leads to the suppression of *C. trachomatis growth due to the induction of intracellular enzyme - 2,3-dioxygenase indolamine destroying tryptophan, which is necessary for chlamydia reproduction. As a result, this mechanism leads to the disorder of cell wall synthesis. Moreover,* γ-IF boosts the activity of nitrogen oxide forming synthase in macrophages and epithelial cells, which results in releasing of nitrogen and bactericidal effect [12, 14].

For many years, clinicians have been widely using various combinations of etiotropic drugs with immunotropic agents in order to increase the efficiency of CI therapy [2, 3, 18, 19].

It is known, that γ-IF stimulates the functional activity of macrophages due to the intensification of digestive ability of phagocytising cells, complete process of phagocytosis and lytic decomposition of chlamydia. Consequently, the level of γ-IF is one of the key components determining the type of infectious process [4].

Interferon inducers are drugs causing the formation of endogenous interferon. They lead to the synthesis of the genuine interferon, which, in contradiction to most widely used recombinant IF, lacks antigenicity. Medications of this group are able to stimulate immune reactivity of organism increasing phagocytosis and antibody production [10].

According to immunologists, intervention into the immune system is possible only in case of correspondent indications, specifically, in case of immune deficiency. Thus, the prescription of such drugs is rational only after additional immunological tests. There is still no consensus on the rationality of combination of etiotropic drugs with immunotropic agents by urogenital chlamydiosis [1].

Research objective: to perform comparative analysis of the results of urogenital chlamydiosis treatment and dynamics of particular immuno-

logical indexes in two groups of patients: exposed to doxycycline monohydrate monotherapy (DM) and combination therapy with doxycycline monohydrate with interferon inducer Meglumini acridonacetas (MA).

Materials and methods

The study included 60 women with urogenital chlamydiosis. The diagnosis was based on clinical data confirmed by PCR method.

Other urogenital infections (UGI) were revealed by means of microscopic, cultural and molecular-biological (PCR) methods. Patients with trichomonas and gonococcal infection and also pregnant women were not included into the study.

The disease prescription constituted: up to 2 months – in 14 (23,3%) patients, from 2 to 6 months – in 19 (31,7%) patients, from 6 months to 1 year – in 16 (26,7%), over 1 year – in 11(18,3%) patients.

The age of patients varied within the range of 18-46 years, there prevailed the group of patients at the age of 21-3o years, the average age of patients constituted 25,4±7,2 years.

Depending on the therapy type, the women were divided into 2 groups. Patients of the first group (DM) received doxycycline monohydrate monotherapy (Unidox Solutab) in the dose 100 mg 2 times a day during 7 days. Patients of the second group (DM+MA) received cycloferon in the dose of 2 ml intravenously 10 times during the course together with doxycycline monohydrate in analogous daily doses.

Doxycycline monohydrate is a broad-spectrum antibiotic. It inhibits synthesis of proteins in the microbial cell breaking the connection of aminoacyl-t-RNA with 30S subunit of ribosomal membrane. It is recommended by the treatment of urogenital chlamydial infection as the drug of choice [5, 6, 22].

Meglumini acridonacetas (Cycloferon) – a 12,5% solution for injections) is a low-molecular interferon inducer with the broad spectrum of biological activity (antiviral, immunomodulatory, anti-inflammatory, etc.). According to the medication instructions, the drug can be used by UGC treatment [10, 16].

The clinical-laboratory control of recovery was performed in 4 weeks after the end of treatment.

The blood sampling in patients for immunological examinations was conducted three times: before treatment, after the course of antibiotics and also upon the control of recovery in 4 weeks.

The phagocytic activity of neutrophils were determined according to their ability to grasp latex particles by counting the rate of active phagocytes (phagocytic index – PI) and average number of bacteria absorbed by one phagocyte (phagocytic number – PN). The metabolic activity of leucocytes was studied by NBT-test – spontaneous and induced [11, 15].

The rate of T-lymphocytes producing gamma interferon in the total pool of T-lymphocytes (CD-3) (Tl-IF) was determined by means of flow cytometry with cytofluorometer Beckman coulter cytomics FC 500 with the automatic hematological analyzer Sysmex XT 2000i (Roche, Switzerland). The γ-IF concentration in blood serum was determined by means of EIA with the immunoenzymometric kit for quantitative determination of human γ-IF in blood serum (Bender Med Systems CmbH, Austria).

Analogous studies were performed in the control group – 30 practically healthy women of reproductive age (average age – 25,8 years).

The research was approved by the Ethics Committee of ISMU, all patients were previously informed about the examination, diagnosis, treatment and confirmed their participation in the study by voluntary written consent.

The statistical processing of obtained results was conducted by means of STATISTICA 6.1. The statistical analysis included Mann-Whitney test. Considering the small size of groups, the average means and standard deviation are presented only for better understanding of obtained results. Differences between compared values were considered statistically significant by $p<0,05$ [13].

Results and discussion

Monochlamydeous infection was registered in 18 (30%) patients, in 42 (70%) patients, *C.trachomatis* was combined with other infectious agents: with *U. urealiticum* - in 25 (41,7%), with *M. genitalium* - in 5 (8,3%), with *M. hominis* - in 18 (30%), with *HPV* - in 15 (25%), with *Candida* fungi - in 7 (11,7%), with *Herpes simplex* – in 4 (6,7%). Clue cells were observed in 10 (16?7%) patients.

The disease duration constituted: up to 2 months – in 14 (23,3%) patients, from to 2 to 6 months – in 19 (31,7%), from 6 months to 1 years – in 16 (26,7%), over 1 year – in 11 (18,3%).

The anamnesis of 1 (1,7%) patient included cystic ovaries, of 2 (3,3%) – frequent cystitis, of 1 – chronic pyelonephritis. Among the examined women, 5 (8,3%) had irregular menstrual cycle, 4 (6,7%) complained about painful menstruations, 5 – about excessive menstruationю

The anamnesis of 36 (60%) women included pregnancy, of 35)58,3%) – delivery, of 5 (8,3%) – miscarriage, in 1 patients there was previously registered missed miscarriage.

Out of 60 patients, 35 (58,3%) presented problems of vaginal discharge, 12 (20%) – of itching, 14 (23,3%) – discomfort in the area of external sex organs. Painful urination was registered in 2 (3,3%) women, lower abdominal pains – in 11 (18,3%), dyspareunia – in 3 (5%) patients.

The examination revealed signs of cervicitis in 56 (93,3%) patients. A typical symptom was cervical discharge, primarily mucoid – in 55 (91,7%).

In 1 (13,3%) patients there was observed purumucuos cervical discharge caused by concomitant infection with *M. genitalium* and aerobic potentially pathogenic microbiota - *Staphylococcus spp., Streptococcus spp.* in high titer (>10^5 CFU\ml).

In four patients by the lack of clinical symptoms of inflammation, there was revealed *C.trachomatis* DNA in the cervical canal.

By microscopic examination of cervical canal discharge, the leukocytic reaction constituted: in 36 (60%) patients – up to 20 cells per field of view, in 17 (28,3%) – from 20 to 40, in 7 (11,7%) – 41-50.

Symptoms of urethritis (hyperemia, urethral sponge edema and mucous discharge) were revealed in 2 women, there was revealed *C.trachomatis* DNA in their urethral material and increase of leucocytes up to 25-30 per field of view.

The analysis of functional activity of peripheral blood neutrophils in comparison with the women with UGC showed the reduction of neutrophil ability to absorb foreign substances (PI), PN before treatment was also low. In the DM group, the spontaneous NBT-test was insignificantly increased, while in the group DM+MA, on the contrary, it had the tendency to decrease, the induced NBT in both groups before treatment was lowered. This indicates low absorbing and metabolic activity of the phagocytic system, h.e. low phagocytic response to the infection (Table 1).

Table 1

Indexes of functional activity of peripheral blood neutrophils in women with urogenital chlamydiosis during doxycycline monohydrate monotherapy and combination of doxycycline monohydrate with Meglumini acridonacetas

Groups	PI (%)	PN	spontaneous NBT (%)	induced NBT (%)
Control group (n=30)	64,87±7,29	5,30±1,83	37,3±6,27	51,0±6,26
DM group before treatment (n=30)	57,73±10,77 p=0,005	4,87±2,10 p=0,237	37,87±6,43 p=0,756	47,23±6,65 p=0,049
DM group after treatment (n=30)	57,63±9,91 p_1=0,836	4,97±2,09 p_1=0,952	39,96±7,71 p_1=0,214	49,47±8,51 p_1=0,201
DM+MA group before treatment (n=30)	54,87±12,62 p=0,001	5,20±2,14 p=0,506	35,03±5,97 p=0,156	43,30±6,15 p=0,00005
DM+MA group after treatment (n=30)	53,27±12,44 p_1=0,668	5,23±1,70 p_1=0,756	35,13±6,11 p_1=0,941	43,27±6,83 p_1=0,976

Note: p – level of statistical significance by comparison of indexes before treatment with the control group; p_1 - – level of statistical significance by comparison of indexes before treatment and after treatment.

After the treatment in the group of patients receiving doxycycline monohydrate monotherapy, the rate of neutrophils got into phagocytosis remained practically the on the same level (Table 1), other indexes of functional activity of neutrophils insignificantly increased: PN – by 1,02 times, spontaneous NBT – by 1,06 times, induced NBT – by 1,05 times.

In the group of patients receiving doxycycline monohydrate with Meglumini acridonacetas, the change of indexes of functional activity of neutrophils after the treatment was identical: PN, spontaneous and induced NBT-test remained practically on the previous level, while PI even slightly decreased (by 1,03 times).

In the general group of patients the rates of Tl-IF in the common pool of T-l and the level of γ-IF in blood serum did not differ significantly from the analogous indexes in the group of healthy people. In this regard, patients were divided into 3 subgroups: 1 subgroup – the level of Tl-IF and γ-IF is increased, 2 subgroup - the level of Tl-IF and γ-IF is normal, 3 subgroup - the level of Tl-IF and γ-IF is is decreased (Table 2).

The analysis of studied parameters in the group of patients receiving doxycycline monohydrate monotherapy showed, that in the 2nd subgroup of patients, both indexes after the treatment and upon control of recovery practically did not change. In the 1st subgroup of patients both indexes increased before the treatment decreased after the treatment up to normal values and remained this level upon control of recovery. In the 3-rd subgroup of patient, both indexes after the treatment and upon control of recovery remained practically on the same level (Table 2).

The analysis of studied parameters in the group of patients receiving doxycycline monohydrate with Meglumini acridonacetas showed, that in the 2nd subgroup, both indexes after the treatment and upon control of recovery practically did not change staying normal. In the 1st subgroup, there was registered slight increase of Tl-IF rate and γ-IF level after the treatment and further re-

duction upon control of recovery, while in comparison with the control group, the studied indexes remained high. In the 3rd subgroup there was registered the increase of Tl-IF rate and γ-IF level after the treatment and upon control of recovery. The difference of the degree of index increase is the following: the rate of T-lymphocytes producing γ-IF grew y 1,96 times after the treatment and by 3,34 times in 4 weeks after the end of treatment. However, in spite of the positive dynamics in terms of Tl-IF, the level of γ-IF in blood serum increased by only 1,3 times after the treatment and by 1,5 times upon control of recovery (Table 3).

Table 2

Rate of Tl-IF and level of γ-IF in peripheral blood of UGC patients during doxycycline monohydrate monotherapy

Indexes	Control	General group of UGC patients	Subgroup 1	Subgroup 2	Subgroup 3
	(n=30)	(n=30)	(n=12)	(n=7)	(n=11)
Tl-IF (%) before treatment	4,34±1,94	4,46±3,53 p=0,574	8,03±2,43 p=0,01 p_1=0,0006	3,32±1,51 p=0,278 p_1=1,000	1,29±1,09 p=0,00003 p_1=0,818
Tl-IF (%) after treatment			4,51±0,88 p_2=0,248	3,33±1,32 p_2=0,949	1,31±1,30 p_2=0,006
Tl-IF (%) upon control of recovery			4,21±0,88 p_3=0,003	3,47±1,12 p_3=0,848	1,30±1,12 p_3=0,002
			(n=11)	(n=7)	(n=12)
γ-IF (pg/ml) before treatment	2,67±0,47	2,81±0,89 p=0,631	3,79±0,48 p=0,00001 p_1=0,0007	2,68±0,12 p=0,713 p_1=0,749	1,98±0,39 p=0,0008 p_1=0,194
γ-IF (pg/ml) after treatment			2,73±0,53 p_2=0,555	2,62±0,36 p_2=0,848	2,01±0,50 p_2=0,100
γ-IF (pg/ml) upon control of recovery			2,85±0,56 p_3=0,001	2,61±0,32 p_3=0,701	2,03±0,35 p_3=0,004

Note: p - level of statistical significance by comparison of indexes before treatment with the control group; p_1 – level of statistical significance by comparison of indexes before treatment and after treatment; p_2 - level of statistical significance by comparison of indexes after treatment and upon control of recovery; p_3 - level of statistical significance by comparison of indexes before treatment and upon control of recovery.

By the clinical-laboratory control of recovery, the *C. trachomatis elimination constituted: by* doxycycline monohydrate monotherapy – 100%, by combined therapy – 96,7%. In 1 patient receiving combined therapy, upon control of recovery in 4 weeks after the end of therapy by the lack of clinical inflammatory symptoms there was revealed *C.trachomatis* DNA in the cervical canal by leukocytosis 15-20 cells per field of view. Upon the examination of patient in 2 weeks, the clinical-laboratory picture remained the same. It should be noted, that before the treatment, in patient, there was registered asymptomatic chlamydial infection of cervical canal by analogous leukocytosis. The level of γ-IF in blood serum was reduced to 1,34 pg/ml by the Tl-IF rate reduced to 0,98% in the common pool of T-lymphocytes. In 2 days after the end of treatment, these indexes slightly (and insignificantly) increased up to 1,38 and 1,0% respectively, remaining practically on the same level in the beginning of the 5th week after the treatment upon control of recovery.

After the treatment, the microscopy of urogenital discharge showed the absence of leukocytosis in the cervical canal, urethra and urine in 59 women out of 60.

The comparative analysis of the results of immunological study in the most problematic group of patients – with lowered parameters before the treatment – showed, that the addition of Meglumini acridonacetas to doxycycline monohydrate generally leads to the growth of % concentration of T-lymphocytes producing γ-IF and increase of its level in blood serum. However, the etiological and clinical efficiency of doxycycline monohydrate monotherapy in combination with the immunotropic drug in these groups of patients turned out to be nearly identical: by doxycycline monohydrate monotherapy – 100%, by combined therapy – 91,7% (in 11 out of 12 patients).

Table 3

Tl-IF rate and γ-IF level in peripheral blood of UGC patients during combined tretamnet with doxycycline monohydrate with Meglumini acridonacetas

Indexes	Control	General group of UGC patients	Subgroup 1	Subgroup 2	Subgroup 3
	(n=30)	(n=30)	(n=12)	(n=7)	(n=11)
Tl-IF (%) before treatment	4,34±1,94	4,80±4,45 p=0,751	7,84±5,02 p=0,034 p_1=0,525	4,92±2,51 p=0,616 p_1=0,753	1,07±0,65 p=0,00003 p_1=0,009
Tl-IF (%) after treatment			8,45±5,17 p_2=0,237	4,76±1,0 P_2=0,141	2,10±0,93 P_2=0,010
Tl-IF (%) upon control of recovery			5,63±2,01 P_3=0,453	5,54±0,77 P_3=0,294	3,57±1,05 P_3=0,0002
			(n=10)	(n=8)	(n=12)
γ-IF (pg/ml) before treatment	2,67±0,47	2,73 ± 0,93 p=0,515	3,85±0,43 p=0,00002 p_1=0,650	2,59±0,10 p=0,720 p_1=0,002	1,88±0,40 p=0,00004 p_1=0,004
γ-IF (pg/ml) after treatment			3,95±0,48 p_2=0,002	2,76±0,17 p_2=0,753	2,44±0,37 p_2=0,013
γ-IF (pg/ml) upon control of recovery			2,99±0,41 p_3=0,001	2,79±0,15 p_3=0,012	2,81±0,33 p_3 = 0,0001

Note: p – level of statistical significance by comparison of indexes before treatment with the control group; p_1 – level of statistical significance by comparison of indexes before treatment and after treatment; p_2 - level of statistical significance by comparison of indexes after treatment and upon control of recovery; p_3 - level of statistical significance by comparison of indexes before treatment and upon control of recovery.

Conclusion

Consequently, the results of the current study allow to conclude, that the main chain in the treatment is the etiotropic therapy. In our research, the interferon inducer did not have the expected effective influence on the system of phagocytosis and cytokine segment and did not increase the etiological efficiency of treatment. Moreover, the implementation of this drug significantly increased the duration of therapy and unreasonably increased its price.

References

1. Akovyann V.A. Urogenital chlamydial infection: 25 years later. *Gynecology*. 2004; 6(2): 14-22.

2. Allenov S.N., Ivanov O.L., Lomonosov K.M. et al. Immunological aspects of the use of polyoxidonium in the complex therapy of complicated urogenital chlamydiosis. *Russian Journal of Skin and Venereal Diseases*. 2002; 2: 58-61.

3. Barinov A.N., Plavinsky S.L. Use of immune response modulators by urogenital chlamydial infection. Mathematical modeling of the epidemic process of morbidity with urogenital chlamydia. *Vestnik dermatologii i venerologii*. 2010; 2: 96-100.

4. Batyrshina S.V., Simbirtsev A.S., Yunusova Ye.I. *Principles and technologies of treatment of patients with sexually transmitted infections. Treatment of patients with urogenital chlamydia: Advanced medical technologies.* 2006.

5. Kubanovoi A.A. *Management of patients with sexually transmitted infections and urogenital infections (clinical recommendations).* 2012.

6. Vorobyova N.V., Shipitsina Ye.V., Sokolovsky Ye.V., Savicheva A.M. Dynamics of detection of Chlamydia trachomatis by the treatment of urogenital chlamydial infection with doxycycline (Unidox Solutab). *Difficult patient*. 2006; 5(4): 31-33.

7. Galdava G. Some indices of cellular immunity by urogenital chlamydia. *Theses of scientific works of the IX All-Russian Congress of Dermatovenerologists*. 2005; 2: 72.

8. Glazkova L.K. Practical aspects of persistent chlamydial infection. *Venereologist*. 2005; 2: 4-12.

9. *Гинекология*. Gomberg M.A. Reproductive health and infections caused by Chlamydia trachomatis. *Gynecology*. 2011; 1(13): 13-17.

10. Granitov V.M. Chlamydia. *Medical book*. 2000; 192.

11. Козлов В.А., Борисов А.Г., Смирнов С.В., Савченко А.А. Практические аспекты диагностики и лечения иммунных нарушений: руководство для врачей. Новосибирск: Наука, 2009. – 274 с.

12. Mavrov G.I., Chinov G.P. The role of cytokines in the pathogenesis of chlamydia. *Ukrainian Journal of Dermatology, Venereology and Cosmetology.* 2004; 1: 53-59.

13. Maksikova T.M., Kalyagin A.N., Piven D.V. The effectiveness of the use of dihydroquercetin in persons engaged in health groups. *Siberian Medical Journal*. 2011; 6: 127-130.

14. Plakhova K.I., Volkov I.A., Khayrullin R.F. et al. Methodology of studying molecular markers of individual genetic risk of complications of urogenital chlamydial infection. *Bulletin of dermatology and venereology*. 2011; 6: 41-48.

15. Potemkina Ye.Ye., Pozdnyakova R.Z., Manukyan L.M. *Manual for laboratory clinical immunology.* Moscow: RUDN, 2003.

16. Vyshkovsky G.L., ed. Register of medicines of Russia: Encyclopedia of medicines. 16th issue. M.: RLS; 2008, 2007.

17. Ryumin D.V. Persistent chlamydial infection. *Bulletin of postgraduate medical education.* 2000; 2: 8-15.

18. Sidorova I.S., Aleshkin V.A., Afanasyev S.S. Efficience of complex therapy of urogenital chlamydiosis with the use of immunocorrecting drugs. *Obstetrics and gynecology.* 2002; 4: 38-41.

19. Yanova L.V. *Immunomodulatory therapy in the complex treatment of urogenital chlamydiosis in women.* [Synopsis of dissertation]. St. Petersburg, 2008.

20. Centers for Disease Control and Prevention. Sexually Transmitted Diseases Treatment Guidelines. *Morbidity and Mortality Weekly Report* (MMWR) 2010; 12: 1-114.

21. Haggerty C. L., Gottlieb S. L., Taylor B. D., Low N., Xu F, and Ness R. B. Risk of sequelae after Chlamydia trachomatis genital infection in women. *Journal of Infectious Diseases.*2010; 2: 134–155.

22. Lau C.Y., Qureshi A.K. Azithromycin versus doxycycline for genital chlamydial infections: a meta-analysis of randomized clinical trials. *Sex. Transm. Dis*. 2002; 29(9): 497–502.

23. No authors listed. CDC Grand Rounds: Chlamydia. *MMWR Morbib. Mortal. Wkly Rep.* 2011; 60(12): 370–373.

24. WHO. Prevention and control of sexually transmitted infections: draft global strategy, 2006. Available from: http://www.who.int/reproductive-health/docs/stis_strategy.pdf.

Contacts:
Corresponding author: Malova Irina Olegovna, Doctor of Medical Sciences, Professor, Head of the Department of Dermatovenerology of ATF and TS of Irkutsk State Medical University, Irkutsk.
664025, Irkutsk, ul. Rossiyskaya, 16.
Tel.: (3952) 24-23-13.
E-mail: marinakartina@mail.ru

UDC 616.5-001.1: 615.262: 615.326:615.453.2

CLINICAL SUPERVISION OF THE EFFICIENCY OF THE UNIVERSAL HYGIENIC MEDICATION BY SKIN LESIONS

[1] International medical center "VREMYA", Saint-Petersburg
[2] State Scientific Research Testing Institute of Military Medicine, Saint-Petersburg

N.V. Shperling[1], I.A. Shperling[2]

The article presents high efficiency and usability of the universal hygienic medication "Telokhranitel" on the basis of porous siliceous sorbent with microdoses of argentum and lithium by various skin lesions, including cuts, cat scratches, mechanical injury. After one- or two-time implementation of the hygienic medication, the epithelization of wounds occurred in 4-5 days without further formation of cicatricial tissues and pigmentation. Additional means of treatment were not required.
Key words: *universal hygienic medication, "Telokhranitel", siliceous sorbent, skin lesions.*

Fundamental researches in the sphere of wound process pathogenesis and its peculiarities showed, that irrespective of genesis and wound localization, their regeneration proceeds according to the same biological laws, and the treatment strategy should be determined by the phase of wound process (inflammation, regeneration, cicatrix reorganization). Generally, wound treatment is aimed at the prevention of infection, boosting of detersion and regeneration processing [4, 7, 9].

At the present time, there exists a broad spectrum of wound treatment methods (vacuum treatment, abacterial environment, medical lasers, etc.), while local treatment remains dominant [1, 3, 5, 6, 7].

Dressings by wound treatment in the 1st stage of wound process (sorbents, gel, ointments, composite therapeutic and wet-to-dry dressings) should possess sorption capacity, provide drainage of exudate and microflora from the wound bed, possess antiedemic, anti-inflammatory, antimicrobial, proteolytic activity, prevent resorption of toxins into subjacent tissues, normalize microcirculation and regeneration and if necessary cause local anesthetic, hemostatic effect. The intensity of curative effect is increased by impregnate medicinal agents: chlorhexidine, miramistin, dioksidin, furaginum, lidocaine, sanguiritrin, aminocaproic acid, feracryl, methyluracil, etc. In the 2nd phase of the wound process, there are implied bandages ensuring normal cell proliferation of regenerative tissue beneficial to angiogenesis, displaceability and synthesis of epithelial cells protecting the wound from secondary infection. Dressings implied in the 3rd phase of the wound process should provide the conditions for the formation of regenerative tissue cells and epithelium, protection of wounds from secondary infection, prevention of cicatricial keloid formation.

The growth of the number of pyoinflammatory diseases and purulent compications by soft tissue traumas on the background of the increase of resistance of pyogenous microflora to antibiotics and the reduction of body resistance determine the necessity of creation of new dressing materials with targeted exposure to the course of the wound process, including wound coverings with antimicrobial effect [3, 5, 10].

Strong interest present sorbents containing cluster silver [2] characterized by complex antibacterial, antiviral and antifungal activity.

The objective of the current work is to present the results of clinical implementation of the universal hygienic topically-applied medication on the basis of porous siliceous sorbent with microdoses of argentum and lithium "Telokhranitel" (OOO Pharmaceutical company "Sanat", Novosibirsk; reg. No. of the Declaration of conformance TA № RU Д-RU.AE96.B.01486 of 02.07.2015) by skin lesions. The given medication is intended for first-aid treatment of wounds, rubbing wounds, intertrigos, inflammatory skin diseases and burns, itching and irritation by allergic rashes and insect bites, possess expressed antimicrobial effect, neutralizes toxins. The medication presents finely dispersed white loose powder, nontoxic and noninvasive [8, 11].

Materials and methods

There were observed patients with skin lesions using the universal hygienic medication Telokhranitel immediately after the injury and repeatedly (if necessary) in 1-2 days.

Results and discussion

Clinical case 1. Patient A. (56 years) implied Telokhranitel by cut wounds of distal phalanges of the 2nd and 3rd fingers on the right hand. In 24 hours after the implementation, there was registered the lack of inflammatory reaction of paravulnar tissues, painfulness in the zone of wounds. In 5 days on the locus of injury on the background of unchanged skin there were observed only dry crusts (Figure 1).

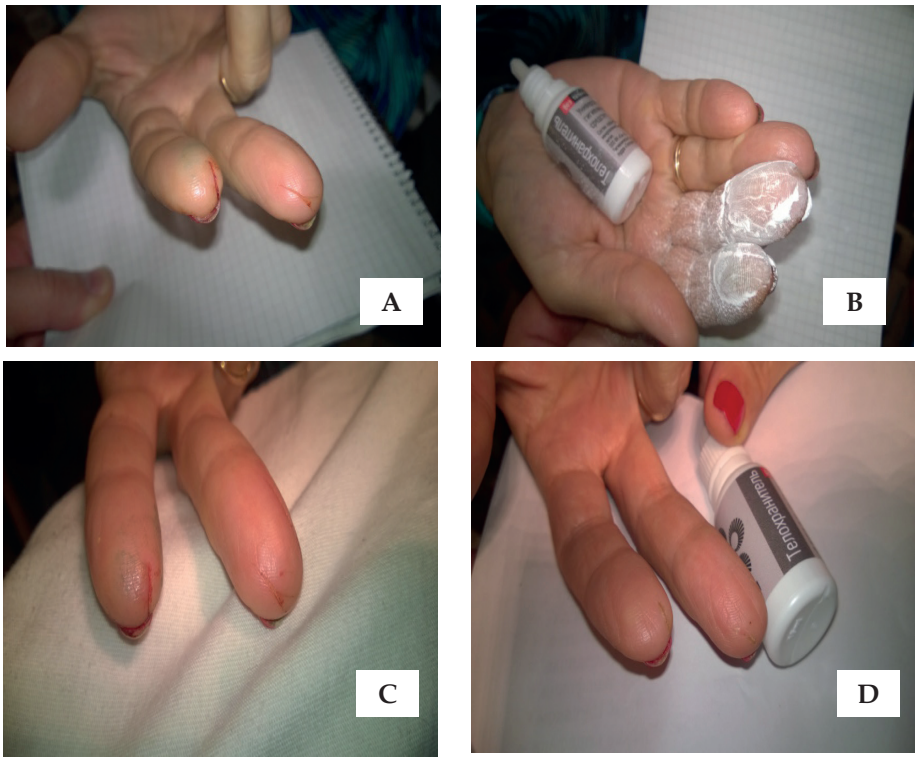

Figure 1.
Cut wounds of distal phalanges of the 2nd and 3rd fingers on the right hand
A – after the injury; B – after Telokhranitel use; C – in 24 hours after Telokhranitel use; D – in 5 days after the injury and double Telokhranitel use

Clinical case 2. Patient B. (57 years) implied Telokhranitel by cat scratch of the left breast. In 4 days after the injury and Telokhranitel use the locus of injury there were observed dry crusts. After complete epithelization, there were observed no cicatricial tissues and pigmentation (Figure 2).

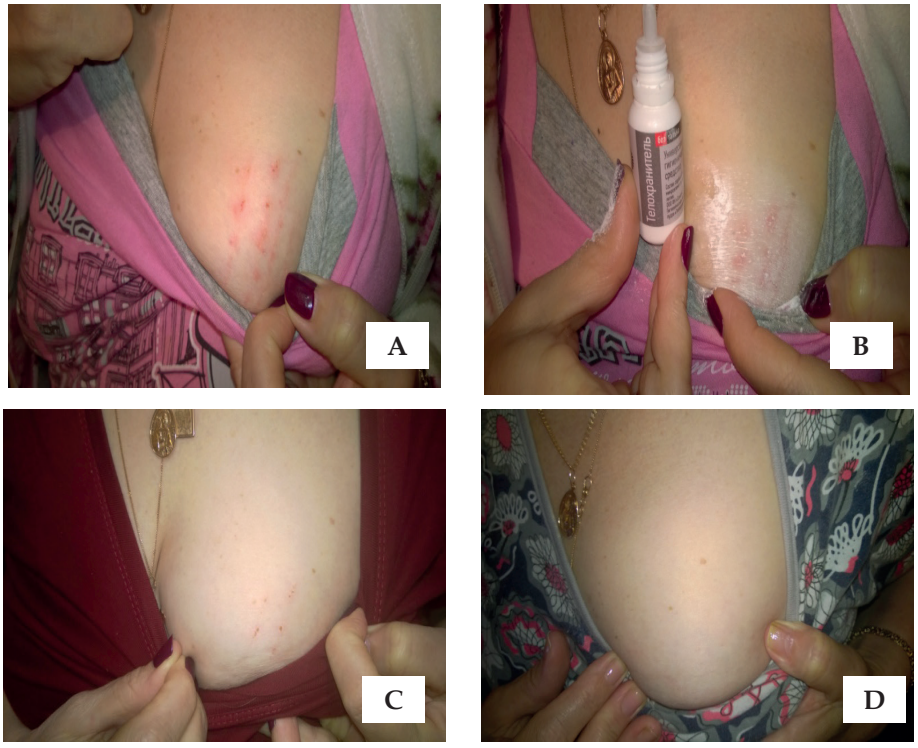

Figure 2.
Cat scratch of the left breast
A – after the injury; B – after Telokhranitel use; C – in 4 days after the injury and one-time Telokhranitel use; D – lack of cicatricial tissues and pigmentation in the zone of injury after regeneration

Clinical case 3. Patient C. (45 years) implied Telokhranitel by mechanical injury of skin of the right thumb. In 3 days after the injury and Telokhranitel use the locus of injury was characterized by the lack of exudation and skin hyperemia; in 5 days there occurred complete epithelization of the injury locas (Figure 3).

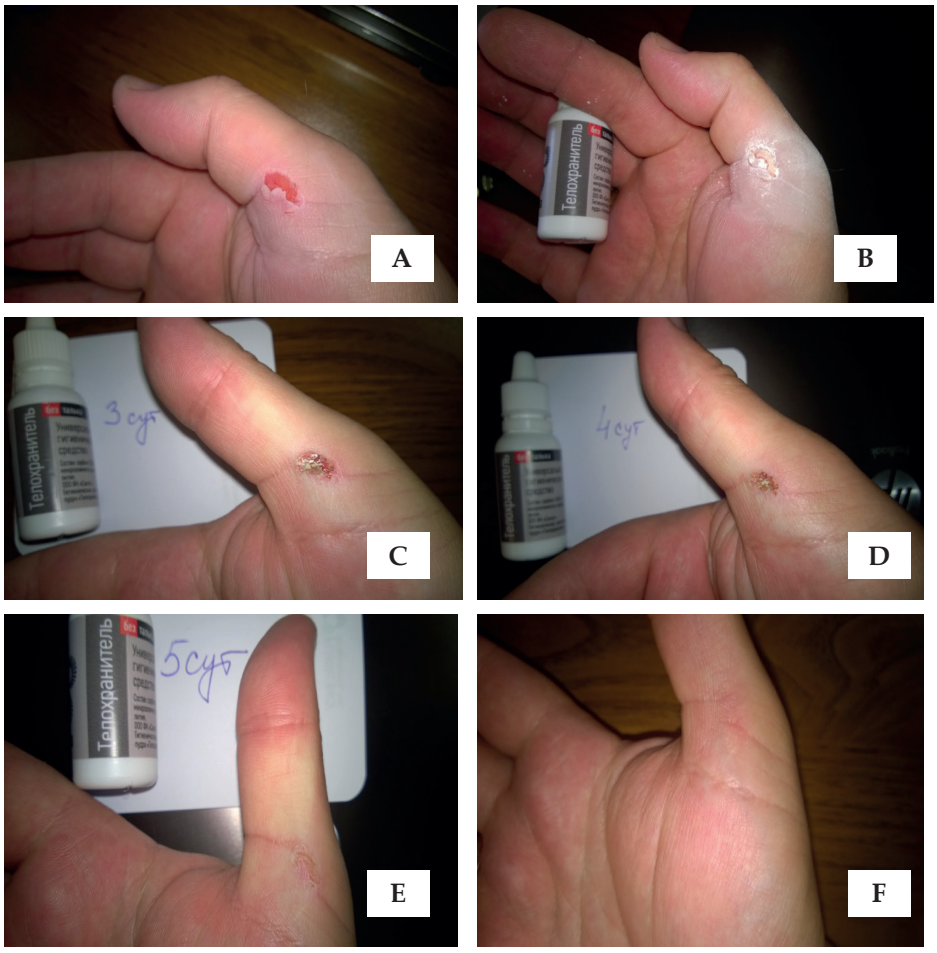

Figure 3.
Mechanical injury of skin of the right thumb
A – after the injury; B – after Telokhranitel use; C, D, E – in 3,4,5 days after the injury and double Telokhranitel use;
F – lack of cicatricial tissues after regeneration

Clinical case 3. Patient C. (46 years) implied Telokhranitel by razor blade cut in the nasolabial triangle zone. In 1 day after Telokhranitel use there lacked symptoms of inflammation, in 6 days – there was reached complete epithelization (Figure 4).

Conclusion

Consequently, the universal hygienic medication "Telokhranitel" on the basis of porous siliceous sorbent with microdoses of argentum and lithium is an afficient and usable means of first aid by skin lesions, including cuts, cat scratches, mechanical injury. After one-time or two-time implementation, the epithelization of wounds occurred in 4-5 days without further formation of cicatricial tissues and pigmentation. Additional means of treatment were not required. The study results substantiate the reasonability of further clinical observations for the elaboration of differentiated approach to the implementation of the given means by skin lesions of various etiology.

References

1. Adamyan A.A., Dobysh S.V., Tyurin B.V. Modern dressings for local treatment of wounds. *Economic bulletin of pharmacy*. 2007; 4: 61-79.

2. Blagitko L.I., Polyakevich A.S., Brombin A.I. *Application of silver preparations in medicine.* Novosibirsk: ZAO "Vektor-Best", 2002.

3. Vinnik Yu.S., Markelova N.M., Solovyeva N.S., Shishatskaya Ye.I., Kuznetsov M.N., Zuyev A.P. Modern wound covers in the treatment of purulent wounds. *Novosti Khirurgii.* 2015; 5(23): 552-558.

4. Yefimenko N.A., Gumanenko Ye.K. ed., *Military field surgery. National leadership.* Moscow: GEOTAR-MEDIA, 2009.

5. Goryunov S.V., Mikhalsky V.V., Romashov D.V. *Hydrogels "Appolo" in the treatment of acute and chronic wounds, thermal and radiation skin lesions, diseases of the mucous membranes*. Moscow: APOLLO, 2014.

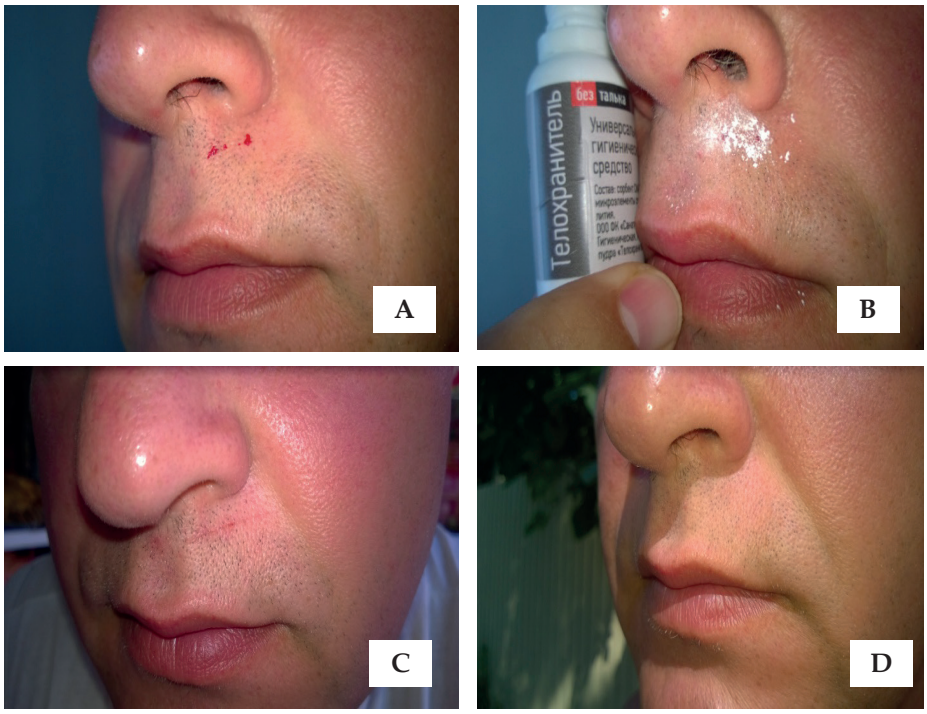

Figure 4.
Razor blade cut
A – after the injury; B – after Telokhranitel use; C – in 1 day after the injury; D – complete epithelization in 6 days and one-time Telokhranitel use

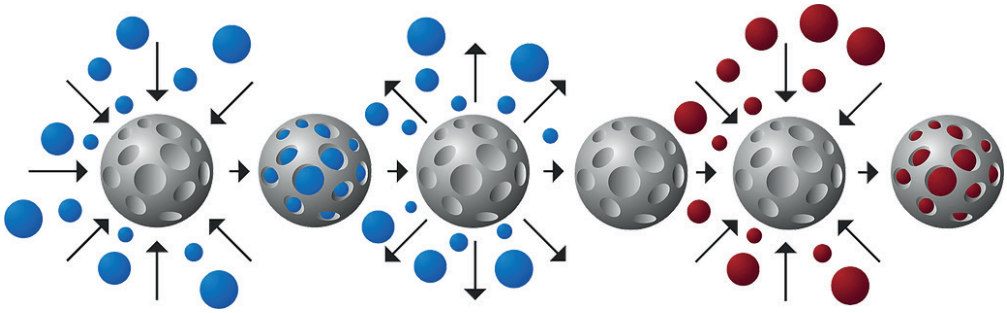

Figure 5.
The mechanism of double action of the universal hygienic medication Telokhranitel sorbent

6. Lazaryan A.D. *Marketing analysis of the consumption of dressings and surgical materials in medical organizations of Stavropol Krai.* [dissertation]. Pyatigorsk, 2016.

7. Nazarenko G.I., Sugurova I.Yu., Gliantsev S.P. *Wound, bandage, patient.* Moscow: Meditsina, 2002.

8. Rachkovskaya L.N., Letyagin A.Yu., Burmistrov V.A., Korolev M.A., Gelfond N.Ye., Borodin Yu.I., Konenkov V.I. Modified sorbents for practical public health. *Siberian Scientific Medical Journal.* 2015; 35: 47-54.

9. Tsybulyak G.N. *General Surgery of Injuries.* Saint-Petersburg: Gippokrat, 2005.

10. Shablin D.V., Pavlenko S.G., Yevlevsky A.A., Bondarenko P.P., Khuranov A.A. Modern wound dressings in *local* treatments of different wounds. *Fundamental research.* 2013; 12(2): 361-365.

11. Available at: https://www.sanatfarm.com/telohranitel [Accessed 02.09.2017].

Contacts

Corresponding author: Shperling Natalya Vladimirovna, Doctor of Medical Sciences, dermatologist of the International medical center "VREMYA", Saint-Petersburg.
194044, Saint-Petersburg, Bolshoi Sampsoniyevsky Prospekt, 60a.
Tel..: (812) 421-75-80.
E-mail: shperling1@yandex.ru

UDC 616.981.71: 616.988.25-002.954.2-07

LABORATORY DIAGNOSTICS OF TISSUE INFECTIONS WITH NATURAL FOCUS (TICK-BORNE RICKETTSIOSIS, IXODIC TICK-BORNE BORRELIOSIS)

[1]Altai State Medical University, Barnaul
[2] "Central Research Institute of Epidemiology" of The Federal Service on Customers' Rights Protection and Human Well-being Surveillance, Moscow

O.V. Beskhlebova[1], V.M. Granitov[1], V.G. Dedkov[2]

The article presents an assessment of diagnostic efficiency of two modern methods of laboratory diagnostics of tick-borne infections – serological and molecular biological. A comparative study of information content of EIA and PCR in diagnostics of tick-borne ricketsiosis (TBR) showed that serological method had the highest frequency (90,0 %) in confirming of clinical diagnosis TBR. The diagnostic value of PCR in the detection of DNA of Rickettsia in skin biopsy specimens was 81,5%. The effectiveness of this method in the detection of DNA of Rickettsia in serum was 11,8%, in whole blood – 5,5%. Immunochip method for the diagnosis of ixodic tick-borne borreliosis showed the maximum diagnostic value on the third week of the disease. The study of paired sera, taken with an interval of 7-10 days, increased its diagnostic efficiency by 30,6%. The study by PCR, using serum and blood from patients, had a low diagnostic value and cannot be recommended as a routine method for diagnosis of ixodic tick-borne borreliosis

Key words: *tick-borne rickettsiosis, ixodic tick-borne borreliosis, enzyme-linked immunosorbent assay, polimerase chain reaction, an immunochip.*

Since the beginning of the 21st century, a high incidence of tick-borne infections with natural foci has been observed in the territories of various regions of the Russian Federation [1, 2].

According to the Federal Service for Supervision of Consumer Rights Protection and Human Wellbeing in 2016, in Russia, the structure of incidence of tick-borne infections is dominated by ixodic tick-borne borreliosis (ITB) with a morbidity rate of 4.18 per 100,000 population. Further, tick-borne encephalitis (TE) and Siberian tick-borne typhus (STT) are 1.39 and 1.06 per 100,000 population, respectively. The incidence of "new" tick-borne infections in the territory of the Russian Federation is low (human granulocytic anaplasmosis (HGA) - 0.04, human monocytic ehrlichiosis (HME) - 0.01 per 100 thousand population).

By the present day, more than 50 subjects of Russia are endemic for a number of tick-borne infections [3]. The presence of regional features of the prevalence and clinical picture of these diseases dictates the need for their comprehensive study in each of the subjects of Russia. Despite the fact that there are a number of works that reflect the clinical picture and laboratory diagnosis of this group of diseases in the context of regional pathology, the principal issues concerning the prevalence and structure of tick-borne infections in the country remain relevant [3]. To address these issues, it is necessary, first of all, to improve the quality of diagnosis of tick-borne natural focal infections.

Diagnostics of STT in endemic regions is based, as a rule, on clinical and epidemiological data, and in typical cases does not cause difficulties [4]. However, the possibility of an atypical course of the disease, the presence of conjugated areas and the marked similarity of clinical manifestations with other rickettsia of the group of tick-borne spotted fevers (TSF) dictates the need for laboratory verification of the diagnosis.

Currently, in the territory of the Russian Federation, laboratory diagnosis of STT is carried out on the basis of approved methodological documents [5], where only serological examination methods based on the detection of R. sibirica antigen are regulated. Unfortunately, they are unsuitable for the species-specific verification of other rickettsia (for example, R. heilongjiangensis), since the rickettsia of the TSF group are characterized by cross-sectional serum reactions [6; 7; 8; 9].

In the presence of pathognomonic syndrome - migrating erythema, as well as a characteristic epidemiological anamnesis (stay on an endemic territory, the fact of tick sucking, seasonal compliance), the diagnosis of an ITB is unquestionable [10; 11]. Nevertheless, the widespread use of non-erythritic forms of ITB and the emergence of clinical manifestations of ITB in any sequence and combination dictates the need for using laboratory diagnostic methods. Despite the variety of laboratory methods, in Russia there are currently no unified algorithms and criteria for laboratory diagnosis of ITB at different stages of the disease, which undoubtedly requires standardization and improvement of the verification of the diagnosis of ITB.

Research objective

To determine the features of laboratory diagnostics of tick-borne natural focal infections (Siberian tick-borne typhus, ixodic tick-borne borreliosis).

Research tasks

1. Verify the diagnosis of tick-borne infections by laboratory methods with the simultaneous study of several biological materials from patients.

2. To evaluate the diagnostic efficacy of serological and molecular biological methods in the diagnosis of STT and ITB.

3. Conduct a comparative analysis of the diagnostic significance of these research methods.

Materials and methods

During 2013-2016, there were examined 213 patients hospitalized in the infectious department of FSBHI "City Hospital No. 5" in Barnaul, Altai Krai with various diseases transmitted by ixodic ticks. The study was approved by the local ethics committee of the the Altai State Medical University of the Russian Ministry of Health.

In addition to clinical and laboratory tests, all patients underwent molecular biological and serological studies.

Verifying methods of laboratory diagnostics were performed on the basis of the "Central Research Institute of Epidemiology" of The Federal Service on Customers' Rights Protection and Human Well-being Surveillance (FBSI CRIE), Moscow.

Material for molecular biological methods was whole venous blood, paired blood serum of patients, collected on the first day of admission to hospital (4.7 + 0.2 day of the disease) and in dynamics after 7-10 days.

Biopsies (crusts) from the place of primary affect, in cases when their preparation was possible, were taken with sterile tweezers on the 8 ± 0,3 days of the disease and before the study were stored in 70 ° ethyl alcohol in the freezer at minus 20°C..

Nucleic acids were extracted individually from each sample of whole blood and serum, as well as from biopsy samples, using the AmplePrime RIBO-prep kit (FBSI CRIE) in accordance with the manufacturer's recommendations [12].

The detection of the TE virus, Borellia burgdorferi sl, Anaplasma phagocytophilum, Ehrlichia chaffeensis / Ehrlichia muris was carried out after the preliminary extraction of nucleic acids by polymerase chain reaction (PCR) in real time with the help of the set of reagents AmpliSens TBEV, B.burgdorferi sl, A. phagocytophilum, E.chaffeensis / E.muris-FL» (FBSI CRIE) according to the protocol recommended by the manufacturer [13].

The detection of rickettsia of the TSF group was carried out by real-time PCR, proposed by J. Stenos et al. [14] in the modification of the FBSI CRIE. Subsequent typing of rickettsia in positive samples was performed by confirming PCR and sequencing of two fragments of gltA citrate synthase genes and OmpA outer membrane protein. Sequencing of the resulting fragments was carried out using an automatic capillary sequencing device "ABI-Prism 3500 XL device" (Applied Biosystems, USA). The resulting sequences were compared with the reference sequences of R. spp., presented in GenBank NCBI and based on their maximum homology, their species identity was determined.

The material for serological testing was paired sera obtained from the venous blood of the patient. The first serum was taken on the day of admission to hospital (4.7 + 0.2 day of illness), the second - after 7-10 days. Detection of specific IgM and IgG antibodies to the agents of HME and HGA by the enzyme immunoassay (EIA) was carried out by commercial immunoenzyme test systems "HME-EIA-IgM", "HME-EIA -IgG" and "HGA-EIA-IgM", "HGA-EIA -IgG" produced by LLC "Omniks", (St. Petersburg).

Detection of specific antibodies of IgM and IgG class to the causative agent of TE was carried out by commercial immunoenzyme test systems "TBEvirus (FSME) IgM EIA" and "TBEvirus (FSME) IgG EIA" (Humburg, Germany). In connection with the presence of a cross-serological reaction between the rickettsia of the TSF group, specific IgM and IgG antibodies to the TR pathogen were determined in the "Rickettsia conorii EIA IgM / IgG" test system (Vircell, Spain) according to the manufacturer's instructions.

The sera were tested for the presence of antibodies to tick-borne borreliosis pathogens with the help of a set of "ImmunoChip Borreliosis" reagents produced by the FBSI CRIE of Rospotrebnadzor. This set of reagents allows for differentiation in a single treatment to differentiate a large spectrum of IgG and IgM antibodies against antigens of Borrelia afzelii and Borrelia garinii, the main pathogenic genes widely distributed in Russia.

In 28 (20.1%) cases TR was diagnosed on the basis of clinical and epidemiological data (sucking and/or crawl of a tick, presence of feverish intoxication syndrome, characteristic exanthema and the primary affect and regional lymphadenitis). In other cases (79.9%), TR diagnosis was established on the basis of the DNA typing of rickettsia in various biological materials from patients (skin biopsy, whole blood clot, blood serum) and/or the detection of antibodies of IgM class by EIA in paired sera increase of seropositivity coefficient, or only in the second serum with negative results in the first serum. In this case, all patients with TR had no markers of other tick-borne infections.

Diagnosis of ITB in 10 (21,7%) people was established on the basis of epidemiological history (sucking or crawling of the tick), and the presence of erythema 3-5 or more centimeters in diameter on the body. In the remaining 36 (78.3%), the diagnosis was confirmed serologically by the determination of antibodies of the IgM class on immunocapses to various antigens of borrelia in paired sera or only in the second serum with negative results in the first. At the same time, all patients with ITB lacked markers of other tick-borne infections.

The diagnosis of TE (4 people), HGA (1 person) is based on the detection of antibodies of IgM class by EIA in the second serum from patients, provided that there are no markers of other tick-borne infections.

The diagnosis of tick-borne mixed infections (23 people) is based on the detection of antibodies of IgM class by the method of EIA (immunochips for ITB) to the target pathogens and/or typing of DNA pathogens in various biological materials from patients (for TR).

Various methods of statistical processing are used in the work, depending on the type of random variables and the research task.

To compare the frequencies of qualitative characteristics in independent samples, the criterion $\chi 2$ was used. In the presence of low frequencies (less than 10), the Yates correction for continuity was used for this criterion. At frequencies less than 5, Fisher's four-field conjugacy tables were used. The relationship between the characteristics was determined using the Spearman rank correlation method.

The level of statistical significance in verifying the null hypothesis was taken as the corresponding $p < 0.05$. The processing and graphical representation of the data was carried out using computer programs Statistica 10.0 (Russified version), Excel 2007.

Results

Diagnosis of hospitalized patients: 139 (65.3%) patients were diagnosed with tick-borne rickettsiosis, including in one case, TE caused by R. heilongjiangensis, 46 (21.6%) - diagnosed with ixodic tick-borne borreliosis, 4 (1.8%) - tick-borne encephalitis, and granulocytic anaplasmosis was diagnosed in one patient. In 23 (10,8%) patients, a mixed infection caused by pathogens of these diseases in various combinations, was detected. By studying the diagnostic significance of PCR and the serological method (EIA), data of the results of the examination of patients who had a laboratory verified diagnosis of TE (110 people) were used. A total of these patients were withdrawn and examined by real-time PCR, proposed by J. Stenos et al. (2005), in the modification of the FBSI CRIE with subsequent typing sequencing of the obtained amplicons of 27 biopsies from the place of primary affect (crusts), 110 whole blood samples (clots) and 110 paired sera collected with an interval of 7-10 days.

In "PCR-positive" patients, Rickettsia sibirica DNA sensu stricto was detected in various biological materials: a biopsy from the place of primary affect, serum of blood, a blood clot.

The study of samples of skin biopsy specimens collected on 8.0 + 0.3 day of the disease (against antibiotics) revealed 22 positive and 5 negative results. The diagnostic significance of PCR in detecting rickettsia DNA in skin biopsy samples was 81.5%.

By the study of serum samples, 13 positive and 97 negative results were obtained, by the study of the whole blood clot - 6 and 104 results, respectively. The effectiveness of the method for detecting rickettsia DNA in serum is 11.8%, in whole blood - 5.5%. Simultaneous study of whole blood and serum samples in TR patients made it possible to increase the detectability of rickettsia DNA only up to 17.3%. It should be noted, that the DNA of rickettsia was found only in the first serum collected on 4.7 + 0.2 day of the disease before the initiation of etiotropic therapy. The presence of significant differences in the frequency of positive results in the PCR biopsy specimen from the place of primary affect and the first blood serum ($p < 0.05$) testifies to the high diagnostic significance of the PCR method when examining biopsies from the site of primary affect. An undoubted advantage of PCR is the possibility of subsequent typing sequencing of the obtained amplicons of the pathogen for the purpose of its species differentiation, which is relevant for regions in which different rickettsia from the group of TSF pathogens are simultaneously encountered.

By the study of serum samples by EIA, antibodies of class Ig M to rickettsia of the TSF group were determined in 99 samples, and 11 samples gave negative results. Thus, the effectiveness of the EIA method for detecting antibodies to rickettsia in the TSF group in patients with CR was 90.0%. In 44 patients (44.4%), Ig M antibodies to rickettsia of the TSF group were detected in the first serum. The study of paired sera collected at an interval of 7-10 days, made it possible to increase the detectability of Ig M class antibodies by 55.6%.

Comparative analysis of the results of EIA and PCR of blood serum in patients with verified diagnosis of TR showed that the coincidence of positive results occurred only in 10 (9.1%) cases. In this case, there are significant differences in the frequency of positive results when using EIA in comparison with PCR and there is no correlation between them ($r = 0,1$).

The obtained data testify that the EIA method in the diagnosis of CR is sufficiently effective under the condition of the study of paired sera taken with an interval of 7-10 days.

Features of laboratory diagnostics of tick-borne borreliosis.

In the period from 2013 to 2016, 46 patients with ixodic tick-borne borreliosis were under observation, including 10 people with an arterial form (21.7%).

Detection of Borrelia DNA in blood serum of patients was carried out by real-time PCR method using the set of reagents "AmpliSens TBEV, B.burgdorferi sl, A. phagocytophilum, E. chaffeensis /

E.muris-FL" (FBSI CRIE). Studies of sera in all patients gave negative results, which does not contradict the literature data on the low sensitivity of PCR in the study of this biological material [15; 16].

Taking into account the absence of non-erythematous forms of the disease, as well as the low sensitivity of PCR, the establishment of a diagnosis of ITB only on the basis of clinical and epidemiological data presents certain difficulties and requires the examination of patients by serological methods.

The serological examination of the patients included the study of paired sera collected on the first day of patients' admission to the hospital and later on in dynamics - after 7-10 days, by means of the immunochip method using the "ImmunoChip Borrelios" diagnostic system produced by the Central Research Institute of Epidemiology of Rospotrebnadzor. This method allows detecting antibodies of Ig M and Ig G class to various antigens of Borrelia.

In cases of diagnosis on the basis of clinical and epidemiological data (21.7%), the first blood sera were collected on the day of admission to hospital, which corresponded to 2.0 + 0.3 day of the disease and in dynamics - on the 7-10th day of inpatient treatment (9.6 + 0.5 day sickness).

In 36 patients (78.3%), the diagnosis was confirmed serologically - the Ig M class antibodies to various Borrelia antigens: in paired blood sera - in 25 people (64.4%), and in 11 people (30.6%) - only in the second serum. In patients in whom Ig M antibodies were detected already in the first serum, it was withdrawn on 8.1 + 1.1 days of illness (i.e., in the second week of the disease). In the absence of antibodies of Ig M class in the first serum, but their presence in the second serum, the first serum was withdrawn in the first week of the disease (2.6 + 0.3 day of the disease) ($p < 0.05$). The second serum of blood in patients with serological confirmation of the diagnosis was withdrawn on 21.4 + 1.4 days of illness (i.e. at the end of the third week of the disease), i.e. later than in patients without serological confirmation of the diagnosis (9.6 + 0.5 day of the disease), ($p < 0.05$). This is due to the length of stay of patients in the hospital.

Thus, the serological diagnosis of ITB with the determination of antibodies to various antigens of Borrelia by the method of immunochips is effective in the collection of biological material from the second week of the disease. If this condition is met, the method of paired sera makes it possible to increase the detectability of Ig M by 30.6%. The highest frequency of positive results is observed at the end of the third week of the disease (21.4 + 1.4 days of illness).

Discussion

An integrated approach to conducting a survey of patients with tick-borne infections with the simultaneous use of molecular genetic and serological methods of investigation made it possible to verify the diagnosis of tick infections in all 213 patients. Due to a comprehensive examination of patients for the full range of tick-borne infections, it was found that the simultaneous infection of individuals with a tick attack by several pathogens is 10.8%.

A comparative study of the informativity of the two modern laboratory diagnostic methods, EIA and PCR, in the diagnosis of CR showed that the serological test (EIA) confirmed the clinical diagnosis with the greatest frequency (90.0%). At the same time, the study of paired sera collected at an interval of 7-10 days made it possible to increase the detectability of Ig M class antibodies by 55.6%.

The low efficiency of the PCR method in detecting rickettsia DNA in blood serum (11.8%) and in whole blood (5.5%), the possibility of detection of the pathogen only in the first serum collected before the etiotropic therapy does not allow to recommend this method as routine diagnosis of TR.

The diagnostic significance of PCR in detecting rickettsia DNA in skin biopsy samples was 81.5%. The presence of significant differences in the frequency of positive results when studying the PCR biopsy from the place of primary affect and the first blood serum ($p < 0.05$) testifies to the high diagnostic significance of the PCR method in the study of biopsy specimens even when etiotropic therapy is performed at 8.0 ± 0.3 day of illness.

The diagnosis was confirmed serologically in 78.3% of all patients with ITB. The study of paired sera made it possible to increase the detectability of Ig M by 30.6%. In patients with Ig M class antibodies already detected in the first serum, it was significantly relieved later (by 8.1 + 1.1 days of the disease) than in patients without serological confirmation of the diagnosis (2.0 + 0, 3 day of illness). In all cases of ITB, in serum collected at the end of the third week of the disease (21.4 + 1.4 days of the disease), specific Ig M antibodies to Borrelia were detected.

None of the patients with a serologically confirmed diagnosis of ITB by PCR in real time in the serum did not detect Borrelia DNA. Thus, the method of immunochips can be recommended as the main method of confirming the diagnosis of ITB. This method shows its effectiveness in the study of sera from the second week of the disease. The maximum diagnostic value of serological examination is observed in the third week of the disease. The study by the PCR method of sera and whole blood from patients can not be recommended as a routine method of diagnosing ITB.

Conclusions

1. Laboratory diagnosis of tick infections requires the obligatory use of several methods of research at the same time.

2. For laboratory diagnosis of TR, the leading method is EIA, which allows to confirm the diagnosis in 90.0% of patients.

3. The PCR method for TR diagnosis shows the greatest diagnostic efficiency in the study of skin biopsies from the place of primary affect (81.5%).

4. The method of immunochips is an effective laboratory method for the diagnosis of ITB, provided it is used from the second week of the disease.

References

1. Aitov K. *Natural-focal transmissible tick-borne infections of the Baikal region.* [synopsis of thesis]. Irkutsk, 2005.

2. Lobzin Yu.V. et al. ed., *Ixodic tick-borne borreliosis in children and adults: methodological recommendations for physicians.* Saint-petersburg, 2010.

3. Maleyev V.V. Review of guidelines for the diagnosis of tick-borne bacterial diseases in Europe. *Clinical Microbiology and Antimicrobial Chemotherapy.* 2005; 7(2): 130–153.

4. Granitov V., Shpynov S., Beshlebova O.et al. New evidence on tick-born rickettsioses in the Altai region of Russia using primary lesion, serum and blood clots of molecular and serological study. *Microbes and Infection*. 2015; 17: 862-865.

5. Yastrebov V.K. et al. *Epidemiological surveillance of tick-borne rickettsiosis. Immunodiagnosis of the disease and methods for identifying the pathogen: guidelines.* Omsk, 1992.

6. Abramova N.V., Rudakov N.V., Penjevskaya N.A. Approbation of enzyme-linked immunosorbent assay for serologic diagnostics of the infections caused spotted fever group rickettsiae. *Epidemiology and Vaccinal Prevention.* 2010; 1(50): 17–21.

7. Shpynov S.N., Rudakov N.V., Samoylenko I.Ye. Genetic identification of the rickettsia groups of tick spotted fever isolated in the foci of tick-borne rickettsiosis. *Jour. Microbiology, epidemiology and immunobiology.* 2004; 5: 43–48.

8. Medyannikov O.Yu. Clinico-epidemiological characteristics of tick-borne rickettsiosis caused by R. heilongjiangensis in the Far East. [synopsis pf thesis]. Moscow, 2004.

9. Brouqui P., Parola P., Fournier P.E., Raoult D. Spotted fever rickettsioses in southern and eastern Europe. *FEMS Immunol Med Microbiol.* 2007; 49(1): 2–12.

10. Lipov A.V., Tovmasyan V.E., Koroi P.V. Lyme disease: literature review and clinical case. *Bulletin of a young scientist.* 2017; 2: 39–47.

11. Dessau R.B., A.P. van Dam, Fingerle V. et al. To test or not to test? Laboratory support for the diagnosis of Lyme borreliosis. *Clin Microbiol Infect*. 2017; - pii: S1198-743X(17)30488-3. - doi: 10.1016/j.cmi.2017.08.025.

12. Instructions for the use of a kit of reagents for extraction of RNA / DNA from the clinical material "AmpliPraim RIBO-prep". 2012.

13. Instructions for the use of a kit of reagents for the detection of RNA / DNA pathogens of infections transmitted by ixodid tick TBEV, Borellia burgdorferi sl, Anaplasma phagocytophilum, Ehrlichia chaffeensis / Ehrlichia muris, in a biological material by polymerase chain reaction (PCR) with hybridization-fluorescent detection "AmpliSens" TBEV, B. burgdorferi sl, A. phagocytophilum, E. chaffeensis / E. muris-FL» AmpliSens [electronic resource]. FBSI "Central Research Institute of Epidemiology" of the Russian Federation. Moscow, 2012.

14. Stenos J., Graves S.R., Unsworth N.B. A highly sensitive and specific real–time PCR assay for the detection of spotted fever and typhus group rickettsiae. *Am. J. Trop. Med. Hyg*. 2005; 73 (6): 1083–1085.

15. Goodman J.L., Bradley J.F., Ross A.E. et al. Bloodstream invasion in early Lyme disease: results from a prospective, controlled, blinded study using the polymerase chain reaction. *Am. J. Med*. 1995; 99: 6–12.

16. Benach J.L., Bosler E.M., Hanrahan J.P. et al. Spirochetes isolated from the blood of two patients with Lyme disease. *N. Engl. J. Med*. 1983; 308: 740–742.

Contacts

Corresponding author: Beskhlebova Olga Vasilyevna, teaching assistant of the Department of infectious diseases and phthisiology of ASMU, Barnaul.
Tel: 9-961-995-04-80.
E-mail: olg.deriglazova@yandex.ru

UDC: 616.5-004:616.5-007.23:615.262:615.355

ENZYME PREPARATION IN THERAPY FOR LICHEN SCLEROSIS IN THE FEMALE EXTERNAL GENITAL ORGANS

Altai State Medical University, Barnaul

Yu.S. Kondratyeva, S.O. Filinova

The article presents literature data on lichen sclerosis localized in female external genital organs. There are described the mesh, disease pathogenesis, features of clinical forms, given modern treatment regimens. There are obtained the results of clinical observation and treatment of lichen, sclerosis of external genital organs, with the "use" enzyme in the form of preparation. The data obtained allow for recommend the state-of-the-treatment of lichen sclerosiss in the processes area in the women.

Key Word: *Lichen sclerosis, genital forms, diagnostics, treatment.*

Lichen sclerosis (*lichen sclerosus et atrophicus*) is a slow onset chronic disease with the visible atrophy of skin and mucous membrane, often localized in the area of female genitals[1].

The disease is of scientific and clinical interest, since an increasing number of patients with lichen sclerosis (LS) is being registered in all countries, and the occurrence of a significant number of cases in girls and women of different ages is of interest to specialists in the clinic, diagnostics and treatment of this pathology [1-4].

LS was firstly described in 1889 by French dermatologist *F. H. Hallopeau,* (1842-1919) [1]. Nosological identity of LS is not clearly defined, however, most authors consider the disease as a kind of superficial scleroderma, some as a variant of lichen ruber planus [1, 2, 4, 5, 6]. Grebennikov V.A. and Chilimova RA (1983) indicated that in lichen sclerosis, in contrast to the atrophic form of the red flat lichen, there is no itching in the disease sites [5]. Some authors consider lichen sclerosis as an independent disease that occupies an intermediate position between the scleroderma and lichen ruber planus, and when localized on genitals it is identified with Kraurosis vulvae [7]. In foreign literature, LS is considered an independent nosological entity [4-6, 8]. Women are exposed to the disease 4-10 times more often than men [3]. Lichen sclerosis can be combined with typical signs of lichen ruber planus and scleroderma lesions. There are described cases of combination with systemic scleroderma [1]. According to foreign researchers, by the localization of LS on the genital organs, the process can be complicated by the development of squamous cell carcinoma [5]. Carlietal P. et al. (2003) observed the association for lichen sclerosis with squamous cell carcinoma against the background of papillomaviral infection [6].

The exact cause of LS is unknown. A number of factors that may be involved in the development of the disease have been examined. In particular, genetic factors: clear link with the family medical history, family cases of LS registered between twins, sisters, mother and daughter and according to the researchers, the development of LS and its degree of severity were connected with the inheritance of a number of genes [7].

Extragenital LS is often combined with morphea, which leads to conclusions about common pathomechanism of these diseases [7]. According to foreign studies, 67 percent of patients with LS showed low titer autoantibodies against the protein extracellular molecule 1 (ESM-1) and collagen XII, resulting in the conclusion of autoimmune disease pathogenesis [9]. Sometimes the progression of LS is associated with local skin damage. The trigger for the progression of LS can be an injury in the area of old scars, radiation exposure of the skin with radiotherapy, roughness, chronic skin irritation with urine in patients with diabetes mellitus [9] Some authors note neuroendocrine mechanisms of the progression of the disease, in particular, dishormonal changes in the pituitary gland- adrenal glands-ovary [8]. Most of the time, LS is observed in girls in the puberty, which may be associated with the low level of estrogen, as a result of later periods of puberty, menstrual dysfunction. Also, the lichen sclerosis process develops against the background of endocrine disorders associated with aging and cessation of the reproductive functions of the body. In women who are in menopausal and post-menopausal periods, the disease manifests itself as a lesion of the vulva and periproctic zone, and takes a persistent recurrent character, resulting in vulvar dystrophy [1]. In the pathogenesis of these disorders, inflammatory diseases of the pelvic organs, an insufficient estrogen level, progesterone and other hormones involved in the synthesis of collagen and other connective tissue components, microcirculation disorder , elasticity and tone of peripheral vessels are important.

The main complaints of LS patients with external genital organs are skin changes, feeling of redensification, tightness, numbness, itching, pain, dysuria, and sexual contacts become painful [3]. The disease sites can be single or multiple, have different shapes-rounded, oval, linear, anoma-

lous, and color- depending on disease state, from bright pink to white yellowish sheen. The surface of the lesion has waxy luster, the peripheral zone is cyanotic. Depending on the stage of the pathological process, the phenomena of induration or atrophy can be made. All these phenomena, in addition to unpleasant subjective esthesia, cause patients considerable moral suffering and significantly diminish the quality of their lives, as they are a serious cosmetic defect.

Eruptions tend to spontaneous resolution, leaving atrophic hypopigmented or amelanotic spots. The disease sites of the genitals can be isolated and characterized by a wide variety of clinical manifestations [7]. The following clinical varieties of genital lichen sclerosis are distinguished: papular, erythematous-edematic, vitiligine, bullous, atrophic and erosive-ulcerative form. Quite often lichen sclerosis of vulva turns into kraurosis vulvae, which is characterized by the progression of sclerosis and loss of tissue mobility, subjectively kraurosis vulvae is accompanied by intense itching [7].

The pathomorphological picture of LS is represented by the atrophy of the epidermis, hyperkeratotic with follicular plugs, and hydropic degeneration of the basal layer. In the dermis, directly under the epidermis, there is edema with the homogenization of gelatinous fibers, under which there is a tramline infiltrate from lymphocytes with an admixture of histiocytes. With a bullous form, a bubble forms in the edema zone, and the infiltration diminished in intensity [7].

The main goal of LS therapy is to slow the progression of the disease, to achieve stabilization of the process, and then regress the clinical picture, so treatment should be timely, multi-component and pathogenetically justified. Considering the probability of development of cicatricial deformity of the mucous genital organs in patients, the analysis of clinical manifestations, outcomes and active search for effective methods of treatment is carried out.

Three pathogenetic links determine the range of curative interventions for the treatment of genital injuries in the case of lesion scleroderma and LS: excessive fibrotic formation, a violation of microcirculation and immune disorders. Currently, anti-bacterial drugs of the penicillin group are used in the therapy of LS: benzylpenicillin, penicillamine (fusidic acid, oxacillin sodium, ampicillin, amoxicillin are used for intolerance), enzymes (lidase, trypsin, chymotrypsin, solcoseryl, hyaluronidases), vasoactive agent (trental, pentoxifylline, xantinol nicotinate), vitamins A, E, B, D2, nicotinic acid. Glucocorticoid ointments, also solcoseryl, actovegin, methyluracil ointments are applied externally to the disease sites . In a subacute stage, physiotherapeutic procedures are prescribed: ultrasound , sonic phoresis, phonophoresis with lidase, photophoresis, electrophoresis , thermal procedures (paraffinotherapy, peloid , diathermy), balneotherapy [9, 10, 11, 12]. A number of modern medicines have the ability to act simultaneously at many pathogenetic links of the LS. These medicines are longidaza, which belongs to the group of enzymatic drugs. The combination therapy of plaque scleroderma includes the usage of longidaza in the form of a solution 3000 IU for intramuscular injection [13, 14]. Due to the peculiarities of the blood supply and innervation of the external genital organs, the stabilization and resolution of the process occurs in the form of a reduction in the local inflammatory pattern, indurative manifestations, trophic disturbances from the medial vulva to lateral areas, and a gradual decrease in itching, burning sensations from the lateral areas to the clitoral and the anterior commissure [12, 13]. The results of biochemical and immunological, histological studies indicate that longidaza does not damage ordinal connective tissue, does not have a teratogenic and carcinogenic effect [14]. Experimental study of the pharmacokinetics of suppositories revealed that the drug is distributed at high speed throughout the body, absorbed well into the bloodstream and reaches a maximum concentration in the blood after 1 hour, with a characteristic lack of tissue cumulation. The elimination half-life is 42 to 84 hours. Bioavailability of longidaza suppositories is at least 70% [14]. When prescribed in combination with other drugs, the possibility of increasing their bioavailability and enhancing systemic action should be considered.

We have the clinical experience of using longidaza suppositories in therapy of patients with LS.

Materials and methods

At the Department of Dermatovenereology, Cosmetology and Immunology, Altai State Medical University, 16 women were diagnosed with a diagnosis of LS of external genital organs. The age of the patient ranged from 30 to 65 years. The duration of the disease ranged from 2 to 15 years.

Patients at circulation noted the following complaints: skin changes in the genital area were observed in 15 women (93, 75%), 8 women (50%) noted the feeling of redensification of the skin and mucous external genital organs, a feeling of tightness and numbness disturbed the same number of patients - 8 women (50%), itching was present in 10 patients (62.5%), discomfort and soreness in sexual intercourse - 5 women (31.25%). At the same time, 2 patients (12.5%) had localization of LS lesions on the skin.

In studying the medical history of life, concomitant somatic diseases were noted: 10 women (62,5%) had bronchopulmonary diseases: acute respiratory diseases- tonsillitis- 6 women (37.5%), strep throat- 4 women (25%); also there was a pathology of the endocrine system: 3 women (18.75%)

had hypothyroidism and 1 woman (6.25%) had diabetes mellitus. There were 5 women in menopause (31.25%).

The clinical picture in patients was represented by similar symptoms- multiple and / or single lesions, depending on the disease state with varying degrees of color, atrophy and sclerosis. All of the patients received treatment: anti-infectives of the penicillin, vasoprotective (pentoxifylline, xantinol nicotinate), drugs that improve microcirculation and have reparative properties (actovegin, solcoseryl), vitamin and microelement complexes. All women in the combination therapy were prescribed an enzyme preparation of longidaza 3000 IU vaginally once in 2 days - 10 suppositories. External therapy included the usage of topical glucocorticoids of weak activity in combination with actovegin or solcoseryl.

The effectiveness of treatment after 1 month was evaluated according to the following clinical score: the dynamics of the pathological process, taking into account the local inflammatory pattern, inducible manifestations and trophic disorders (discoloration, decreased dryness of the mucocutaneous membranes, the disappearance of inflammatory infiltration and epithelialization of cracks), as well as the reduction or disappearance of itching and burning sensation. The dynamics of the clinical picture are reflected in Figure 1. After the treatment, such clinical signs as inflammatory infiltration, itching, burning sensation were completely stopped in all women, dry skin and mucous membranes were preserved in 2 women (12.5%), the appearance of new cracks was observed in only 2 patients (12.5%).

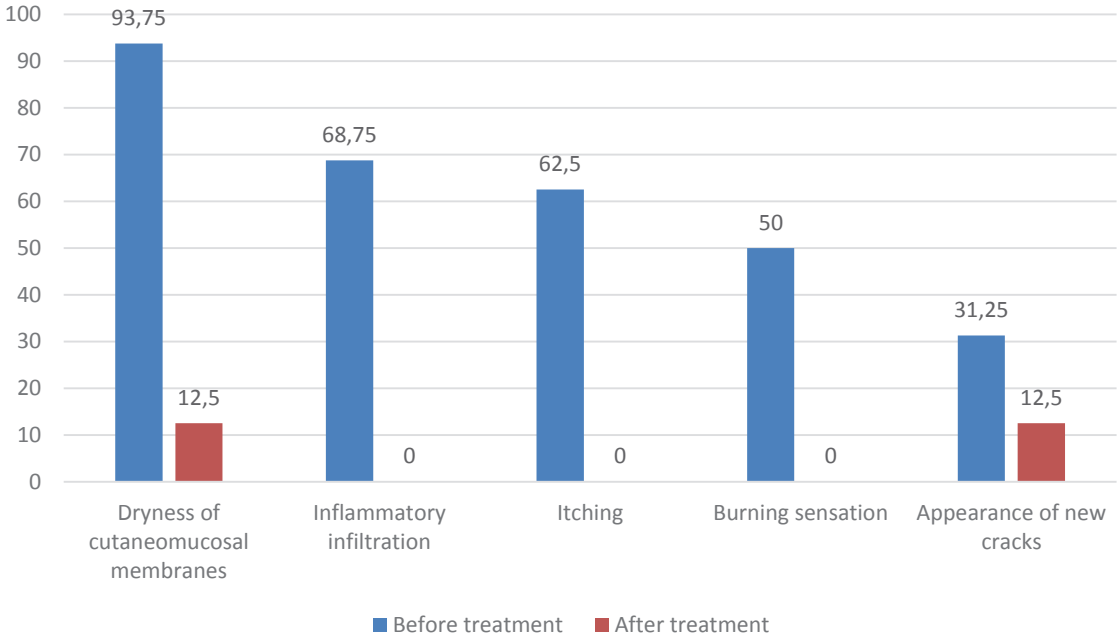

Figure 1.
Clinical features of the LS of the external genital organs

Results and discussion

Clinical observation 1. Patient M., 52 years old, considers herself ill for 12 years, when she first discovered the appearance of spots on the skin of the breast, and then after 4 years she noticed excessive dryness and cracks in the large and small vulvar lip, accompanied by itching, discoloration and structure change of the skin. The gynecologist diagnosed the following: The vulva-vaginal candidiasis. The treatment assigned did not have a positive dynamic. From medical history: Physiological menopause from 43 years. Of the comorbid condition- idiopathic hypertension, hypothyroidism, medically compensated.

St.localis: The pathological process is widespread and localized on the skin of the chest, in the area of the external genital organs with the transition to the periproctic zone. There are several lesions up to 3 cm in diameter, oval in shape and indurated in the brilliantly white colored central part on the skin of the chest. The disease sites of the skin of the external genital organs in the form of sclerosis sites with radial folding of tissues, depigmented spots, cracks, bleeding painful erosions amid hyperemia and atrophy. Also around the affected areas was a pale pink bezel. There were initial signs of stenosis of the vagina. The zone of the atrophic process had the outlines of the "eight" or the shape of the "sand-glass". Subjectively, the patient was troubled by itching, burning sensation, painful urination and dryness of mucous of EGP (external genital pore). The following diagnose was given:

Lichen sclerosis of the skin and external genital organs, erosive-helcoid form. Fixed treatment course: Benzylpenicillin novocainic salt in 600 thousand. Unit I.M. 2 times per day - 20 days, trental endovenous drip No.10, AEvit 1 capsule 3 times a day, suppositories of longidaza 3000 IU vaginally 10 administrations every other day, alternating with suppositories triozhenal No. 10. Apply externaly on the rash ointment solcoseryl and hydrocortisone cream 1%. At the end of therapy, complaints of itching, burning sensation does not note. Clinically, the decrease in the phenomena of atrophy and xerosis, the healing of cracks. After 2 months after treatment, full epithelization of erosions, a decrease in the phenomena of atrophy and sclerosis, the cessation of the appearance of new cracks, the pain symptomatic is stopped.

Clinical observation 2. Patient E., 47 years old, is sick for 3 years, when she first discovered skin changes in the field of EGP, she was not treated, she did not consult doctors. From medical history: a condition of a perimenopause, from accompanying diseases: Diabetes mellitus 2 type.

*St.localis:*on the external genital organs with the transition to the perianal zone, the perineum, areas of atrophy of whitish color against the background of hyperemia and edema of the labia majoria are noted. Subjectively, the patient was bothered by the itching and dryness of mucous EGP, dyspareunia. Diagnosed with: Lichen sclerosis of external genital organs, erythematic form. Fixed the course of outpatient medicine: trental 100 mg 3 times a day 1 month, AEvit 1 capsule 3 times a day, ebastine 10 mg 1 time a day 14 days, suppositories longidaza 3000 IU vaginally 10 administrations every other day, externally- methyluracil ointment and hydrocortisone cream 1%. At the end of the course of therapy, subjective complaints are not noted. In the inspection, reduction of the phenomena of xerosis, atrophy, erythema.

Analyzing all of the above, we can speak about the effectiveness and expediency of combination treatment with the inclusion in the therapy scheme women with atrophic diseases of the external genital organs of the enzyme preparation of longidaza 3000 IU in the form of suppositories. The use of this treatment regimens in our observation allowed us to stop completely inflammatory infiltration, itching, burning sensation in all patients, to reduce the dryness of the cutaneomucosal, and the appearance of new cracks.

Conclusion

Thus, the described changes and the presented clinical examples demonstrate the need for a more careful approach, consultation and treatment of these patients together with gynecologists, endocrinologists. Existing pathological processes in the field of external genital organs in women, of course, cause significant moral suffering to patients and significantly diminish the quality of their lives, which must be remembered when counseling and treating patients with this pathology.

Список литературы

1. Grebennikov V.A., Chilimova R.A. Transformation of scleroatrophic liena into systemic scleroderma. *Vestnik Dermatologii i Venerologii*. 1983; 1(4): 60-63.

2. Kryazheva S.S., Boldyreva M.V. Telangiectatic form of sclerotrophic silicium. *Russian Journal of Skin and Venereal Diseases.* 1999; 1(6): 27—29.

3. *Dermatology: Atlas-reference book: Trans. from eng.: Practice.* 1999.

4. *Bzacco*G. L.etal. Clinical and histologic effects of topical treatments of vulvar lichen sclerosus. Acriticalevaluation. *J. Reprod. Med*. 1993; 38: 37.

5. Chalmers R. J. G. et al. Lichen sclerosus et atrophicus: a common and distinctive cause of phimosis in boys. *Arch. Dermatol*. 1984; 120: 1025—1027.

6. Friedrich E. G., Kalra P. S. Serum levels of sex hormones in vulvar lichen sclerosus and the effect of topical testosterone. *N. Engl. J. Med*. 1984; 310: 488—491.

7. *Private dermatology: Medicine. 1965.*

8. Chfrles C., Clements P., Furst D. Systemic sclerosis: hypothesis-driven treatment strategis. *The Lancet*. 2006; 367(9523): 1683-91.

9. Klaus Wolf, Lowell A. Goldmitt, Stephen and Katz, etc. *Fitzpatrick's dermatology in clinical practice*: in 3 volumes. trans. from the English; Izdatelstvo Panfilova: BINOM. Knowledge laboratory; 2012.

10. Nikitina M.N., Oreshkina Yu.I., Grishko T.N. Focal scleroderma of external genital organs. *Vestnik Dermatologii i Venerologii*. 1983; 8: 39-43.

11. Kulagin V.I., Markina Ye. I. Etiology and pathogenesis of sclerotrophic lichen. *Russian Journal of Skin and Venereal Diseases.* 2003; 1(2):51-52.

12. Sherman V, McPherson T, Baldo M, Salim A, Gao XH, Wojnarowska F. The high rate of familial lichen sclerosus suggests a genetic contribution: an observational cohort study. *J EurAcadDermatolVenereol*. 2010.

13. *Scleroderma: Textbook.* 2002.

14. Vidal reference book: medicines in Russia. Moscow: UBM Medica Rus; 2014.

Contacts

Corresponding author: Kondratyeva Yulia Sergeyevna, Doctor of medical Sciences, Associate Professor, Head of the Department of dermatovenereology, cosmetology and immunology of ASMU, Barnaul.
656038, Barnaul, Lenina Prospekt, 40.
Tel.: (3852) 554578.
E-mail: julia_jsk@mail.ru

UDC 616.314-089.28

OPTIMIZATION OF PROSTHETIC FITTING OF FULL-CERAMIC RESTORATIONS IN DENTISTRY

Altai State Medical University, Barnaul

Grokhotov I. O, Ubrayev R. Ye.

At the present time, all-ceramic restorations are widely used in dentistry. However, the technology of their manufacture is quite complex and requires a lot of a different materials.
One of the serious problems is the fitting of such restorations, especially veneers, which creates certain difficulties in assessing the final quality of the work. In connection with this, we developed a special gel for the fitting of such restorations, which was studied at the experimental stage.
It is established, that the gel is convenient in use, can be totally removed from the ceramic restoration surface, possesses a sufficient fixation strength and does not affect the color and other esthetic characteristics of veneers, which allows recommending it for the study at clinical trials.
Key words: *ceramic restorations, veneers, gel for temporary fixation.*

Introduction

At present, all-ceramic restorations are widely used in dentistry, since they require minimum invasive preparation of the hard tooth tissue. In addition, the excellent properties of all-ceramic restorations, such as color stability, mechanical strength, compatibility with periodontal tissues, as well as pleasing aesthetics, allow them to be recommended for use in most clinical situations [1]. However, the technology of their manufacture is quite complex and requires a lot of a different materials [2]. One of the major problems is the fitting of such restorations, especially veneers, which are often not retained on the tooth, respectively, do not allow to accurately estimate their quality. Although it is very important to estimate the fitting quiescence, the marginal fit, the contact points density, the surface relief and the construction color of such restorations in a non-fixed form, and thereby make sure that we offer the patient the best result of orthopedic treatment [3]. We developed a special gel for the fitting of all-ceramic restorations (such as "Variolink II Try in"), but the use of them in Russia is difficult for several reasons:

- the gel is not licensed,
- it is not available in retail,
-its high cost [4].

Therefore, often, dental practitioners are forced to use auxiliary materials that are not designed for these purposes. Such process is incorrect and often inconvenient.

Research objective

In connection with the foregoing, our purpose was the development of a new gel for the fitting of all-ceramic restorations, using known and safe components for the oral cavity, comparable in quality with imported analogues.

Research tasks:

1. Analysis of literature data on the relevance and ways of solving the problem.
2. Development of the gel composition for the fitting of all-ceramic restorations.
3. Experimental approbation of the developed composition on the laboratory models.
4. Laboratory development of the final version of the optimal gel composition and its practical implementation.

Materials and methods

In order to determine the relevance of the problem, we conducted a survey of dentists who are engaged in the manufacture of ceramic restorations to determine what means they use to sample them, and also what problems they encounter during this stage.

The methods of investigation included in the experimental stage:

- checking the purity of the restoration after flushing the gel from its surface by staining the indicator;
- checking the adhesion of the restoration with the tooth during the fitting with the applied gel;
- determination of the color of the restoration with the applied gel in comparison with the analog and the initial state by means of hardware color determination with the Vita EasyShade instrument;
- determination of the general appearance of the restoration with gel applied under the control of a dental photograph.

As a means for fitting, we used a gel based on glycerin with a mineral filler.

The object of the study was: experimental models in the form of ceramic restorations, as well as a extracted tooth with ceramic veneer made on it.

Results and discussion

In the course of our research there was held an anonymous questionnaire poll among the dentists in Barnaul. Based on the analysis of its results, it is established that 41% of prosthodontists face problems during the testing of all-ceramic restorations. As for the materials used in this clinical stage, only 19% use "Variolink ll Try in" specifically designed for this purpose. 19% use additional materials not intended for fitting, for example: cream-gel Korega, correcting mass from Speedox, YETI Dental spray, ultrasound gel.

Unfortunately, 21% of dentists do not use any materials for fitting, in their opinion, because of the lack of materials, or their high cost. Thus, only 19% of physicians use specialized means for testing all-ceramic restorations at clinical stages. The overwhelming majority of doctors would like to use a new domestic composition for fitting veneers, if they had the opportunity.

As a composition for the fitting of all-ceramic veneers, we used a water-soluble gel developed by us, including an inorganic filler dissolved in a glycerol base. According to its physicochemical properties, the gel was adapted for use according to the existing analog.

Hereafter, to determine the ease of removing the gel after application, there was given an indication of its flushing from the surface of the restoration under running water after its preliminary application with an exposure time of 5 min. After that, the surface was painted with an indicator, followed by rinsing with a different time interval.

The method showed that the gel is washed off with a gap of 30 seconds completely without residue of the indicator, therefore it can not affect other materials used for adhesive fixation (Figure 1).

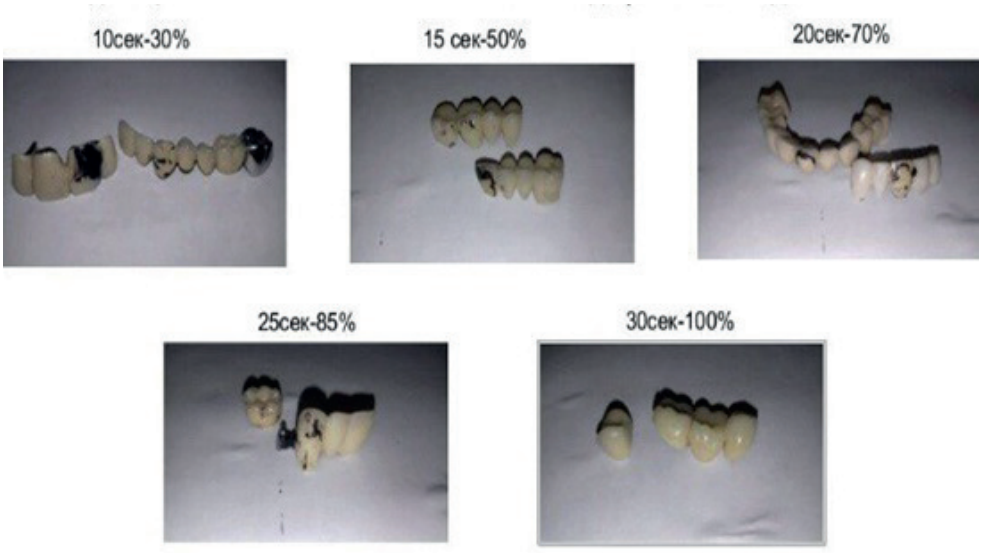

Figure 1. The indication of the purity of rinsing the gel from the restoration surface

The next step was to determine the strength of the gel fixation, for this purpose we compared the developed gel with the analogue "Variolink II try in". The figure provides a photograph that clearly shows the essence of the experiment, which proves that each material is able to keep the veneer from being displaced with an application of a certain weight. The maximum results for the Variolink are 3 grams, for our gel the volume estimates 8 grams. Thus, it can be concluded that the adhesion strength of the gel to tooth surfaces and restoration is sufficient, somewhat exceeding the import analogue.

Further, we carried out a comparative assessment of the color of the restoration on a laboratory model by using Vita Easyshade. To do this, we initially determined the color of the restoration without any materials (result B1 OM3), then we determined the color of the restoration with the Variolink material (result A1 OM3). The gel developed by us showed the result (B1 OM3), which coincides with the initial one and proves the absence of the effect of the agent on the color of the ceramic restoration. This result is very significant, because the color change during the fitting leads to an incorrect evaluation of the final result, and, ultimately, to the patient's negative perception of the work of the dentist.

The analysis of dental photographs showed that our gel does not affect the appearance of the restoration in comparison with the analog ("Varyolink Tray In"), and also when fitting without gel, no does it affect the color of the ceramic veneer and its attachment to the tooth.

Conclusion

Thus, according to the result of the questionnaire, the problem we are studying is relevant for dental practitioners, since most of them in Barnaul use materials that are not intended for fitting

ceramic restorations, or do not use any solutions at all.

The experimental testing of the developed gel allows us to conclude that it is effective and safe.

It is established that the gel is convenient for fitting all-ceramic reconstructions, has sufficient fixation force, does not affect the color and other characteristics of the restoration, which allows an objective assessment of its quality. We plan to continue our clinical research with this gel.

References

1. Kulakov A.A., Gvetadze R.Sh., Krechina Ye.K., Guseva I.Ye. Modern technologies in dentistry. *Bulletin of Roszdravnadzor*. 2009; 6: 55-60.

2. Zholudev D.S. Ceramic materials in prosthetic dentistry. *Actual problems in dentistry.* 2012; 5: 8-14.

3. Vartanov T.O. The basic stages of introduction of technology of metalless designs in the practice of stomatologist. *Siberian medical journal.* 2012; 4: 102-104.

4. Kitayeva T.A., Danilina T.F., Salyamov H.Yu., Verstakov D.V. Modern technologies for making structures from nonmetallic ceramics. *On-line Scientific & Educational Bulletin "Health & education millennium".* 2010; 8: 408-409.

Contacts

Corresponding author: Grokhotov Ilya Olegovich, Candidate of Medical Sciences, Associate Professor of the Department of orthopedic dentistry of ASMU, Barnaul.
656038, Barnaul, Lenina Prospekt, 40.
Tel.: 8-913-023-57-36.
E-mail: ilident@yandex.ru

UDC 618.29:616-053.31-005.4

OBSTETRICAL ASPECTS OF FORMATION OF HYPOXIC-ISCHEMIC CNS DISORDERS IN FULL-TERM LOW-WEIGHT NEWBORNS

[1]Altai State Medical University, Barnaul
[2] FSBHI "Maternity Hospital No.2", Barnaul

N.S. Dolgova[1,2], S.D. Yavorskaya[1,2], L.P. Ananyina[2], Ye.V. Pozhenko[2]

There was carried the retrospective analysis of case records of 100 full-term newborns with diagnosed fetal growth retardation. In order to estimate the predictors of hypoxic-ischemic encephalopathy in newborns there were formed two groups of comparison: newborns without perinatal disorders of CNS (n=49) and with moderate and severe perinatal disorders of CNS (n=51). The comparative analysis was conducted by 125 criteria. It was stated, that prognostically unfavorable factors for the health of newborns are: mother's excessive weight before the period of gestation (21,5±0,5 and 23,8±0,7; p=0,011) and thrombocytopenia shortly before delivery (33,3% and 94,1 %; p=0,011). Pregnant women with markers of placental dysfunction according to the results of Doppler velocimetry (53,1% and 76,5%; p=0,025) in the third trimester of pregnancy constitute the risk group of not only FGR, but also of perinatal CNS disorders.

Kew words: *low birth-weight fetus, fetal growth retardation, newborns, pregnancy, perinatal CNS disorders.*

Introduction

The birth of a healthy child is an invaluable gift that nature gives us free of charge. According to the Federal State Statistics Service, over the past 25 years, infant mortality has declined by 2.4 times (from 17.4 per 1000 live births in 1990 to 7.4 in 2014), but, despite the positive dynamics, this indicator remains 2-3 times higher than in many European countries [1]. It is known that the health of the fetus and the newborn depends largely on the mother's maternal and reproductive health, the course of pregnancy and childbirth [2].

One of the serious complications of gestation is the intrauterine growth restriction (IGR). This complication increases the risk of perinatal mortality by 6-10 times in comparison with newborns born with normal body weight. Children with IGR are at high risk for disorders of neurological and physical development, metabolic syndrome in adulthood, type 2 diabetes, coronary heart disease and hypertension [4, 5, 6, 7, 8]. Obstetricians and neonatologists are well aware that children born with a low body weight present a different contingent of patients. Some newborns are diagnozed with perinatal pathology, while others do not have functional disorders of the central nervous system and are let home from the maternity hospital [9].

Research objective

To establish anamnestic and gestational features of mothers who gave birth to full-term babies with perinatal CNS disorders.

Materials and methods

There was performed a retrospective analysis of 100 delivery records of pregnant women, 100 birth histories and 100 records of newborns born at the term of $37^{0/7}$-$41^{6/7}$ weeks, who had been diagnosed with IGR at birth.

Inclusion criteria: full-term pregnancy, newborn with an IGR, urgent birth with one fetus, parents of a European race.

Exclusion criteria: premature birth, twins, parents of non-European race.

The gestation period at the time of delivery was established by the date of the last menstruation and data of the ultrasound investigation of the fetal egg in the period of 11-14 weeks [10]. The diagnosis of IGR was established after evaluating the physical development of the newborn according to the table of G.M. Dementyeva, taking into account the gestational term [11].

In the course of study, all newborns were divided into 2 comparison groups: the first group consisted of 49 newborns without significant CNS disorders (were let home), the second group - 51 newborns, with perinatal CNS disorders of medium and severe degree, who needed the second stage of nursing. To establish the predictors of combined perinatal pathology, a comparative analysis was carried out on 125 criteria, which were ranked by groups, taking into account the mother's anamnestic data, the features of the course of pregnancy and childbirth.

Statistical processing of the obtained data was carried out on a personal computer using the Statistica 10.0 and Excel 2007 applications. Different methods of statistical processing were used in the work, depending on the type of random variables and the task of the study [12]. To estimate the normality of the distribution of characteristics, the kurtosis and asymmetry indicators characterizing the shape of the distribution curve were used.

The values of the continuous quantities are given in the form M ± m, where M is the sample

mean and m is the standard error of the mean. The values of qualitative characteristics are presented in the form of observed frequencies and percentages. In cases of normal distribution, as well as the equality of variances, Student's t-test was used to compare the averages. The dispersion equation was estimated by the F-criterion. In the case of distributions that do not correspond to the normal law, as well as for variance inequality, the nonparametric Mann-Whitney U-test was used.

To compare the qualitative characteristics, a nonparametric $\chi 2$ criterion was used. In the presence of low frequencies (less than 10), the Yates correction for continuity was used for this criterion. By frequencies less than 5, Fisher's four-field conjugacy tables were used. The critical level of statistical significance for testing the null hypothesis was assumed to be 0.05. In all cases, bilateral criteria were used.

Results and discussion

The average age of mothers (26.6 ± 0.8 and 27.7 ± 0.8, p = 0.3) did not vary in the groups. There were no differences in the evaluation of data from social and obstetrical gynecological history of mothers of two comparison groups. Such important for the formation of placental dysfunction and IGR as a maternal illness, such as hypertension syndrome (10.2% and 7.8%, p = 0.95), violation of carbohydrate metabolism (6.1% and 7.8%, p = 0.956) and thyroid function (18.4% and 25.5%, p = 0.537), diseases of the urinary system (30.6% and 51%, p = 0.062), also did not significantly differ in the comparison groups. When assessing anthropometric data, it was found that for the same average growth rates of mothers of comparison groups (162.7 ± 0.9 and 162.8 ± 0.8, p = 0.9), the mass-growth ratio was different. Mothers who gave birth to children with a lower health index entered into a pregnancy with a greater body weight (56.8 ± 1.3 and 63.1 ± 2.1, p = 0.012), they initially had a higher body mass index (21.5 ± 0.5 and 23.8 ± 0,7, p = 0.011), which can speak of the available hidden violation of carbohydrate-fat metabolism. A number of authors believe that overweight is a risk factor for an unfavorable outcome of pregnancy [13]. Mothers of children of the second comparison group were significantly more likely to have overweight before pregnancy (14.3% and 37.3%, p = 0.017).

The assessment of the course of pregnancy in patients of the two groups of comparison revealed that in most of the women it was complicated. In the first trimester of pregnancy, each tenth patient of two comparison groups (10.2% and 9.8%, p = 0.79) had clinical and paraclinic signs of threatened miscarriage, one out of five had early toxicosis requiring in-patient treatment (20.4% and 19.6%, p = 0.881). Acute respiratory viral infections in the early stages of pregnancy were carried by every fourth patient of the first and every fifth of the second comparison groups (26.5% and 19.6%, p = 0.559). All this could have a negative effect on the formation of the placental complex and subsequently cause its dysfunction. The second trimester of pregnancy proceeded against the threat of termination of pregnancy in 31% of cases, without significant differences in comparison groups (30.6% and 31.4%, p = 0.893), but vulvovaginitis of non-specific etiology was significantly more common in the group of mothers who gave birth to children with low body weight without perinatal CNS injuries (38.8% and 17.6%, p = 0.033). Mothers of children of comparison groups had acute respiratory viral diseases in the second trimester of pregnancy with practically the same frequency (20.4 and 17.6%, p = 0.923). Ultrasonic markers of IGR in the second trimester of pregnancy were diagnosed in 2% of cases, in comparison groups with the same frequency.

Significant gestational complications of third trimester pregnancy, edema due to pregnancy (20.4% and 23.5%, p = 0.892), the threat of premature birth (16.3% and 19.6%, p = 0.868), preeclampsia (4, 1% and 15.7%, p = 0.11) in the comparison groups did not significantly differ. Interesting data were obtained by studying the hematological indices of red blood of mothers who gave birth to low-weight children. So, with statistically insignificant differences in the mean level of leukocytes, erythrocytes and hemoglobin, the average platelet count in the third trimester of pregnancy was lower in the group of mothers who gave birth to children with perinatal CNS disorders (247.2 ± 64.6 and 135.3 ± 4.4 ; p = 0.006). Thrombocytopenia of mild severity preceding labor in this group was detected 3 times more often (33.3% and 94.1%, p = 0.011). Perhaps this is due to the fact that some mothers of newborns of the second group received low molecular weight heparins during pregnancy in order to correct progressive placental dysfunction. The diagnosis of delayed fetal development during pregnancy is more often established in mothers who gave birth to children with perinatal CNS disorders (32.7% and 43.1%, p = 0.382), which was the reason for prenatal hospitalization. By the assessment of the condition of the placental complex before the delivery, signs of premature aging of the placenta, in particular calcints, are more often detected in pregnant women who gave birth without a perinatal complication from the CNS (65.3% and 45.1%, p = 0.057), which may indirectly indicate their higher readiness for extrauterine life. According to the Dopplerometry data, disorders of the uteroplacental blood flow are significantly more often established, on the contrary, in the pregnant women of the second group (53.1% and 76.5%, p = 0.025). The method of delivery in the compared groups was identical, the frequency of operative labor was high (49.0% and 49.0%, p = 0.845).

In most cases, the newborns of the comparison groups were born without signs of asphyxia, with an Apgar score of 8-10 points (91.8% and 88.2%, p = 0.790). However, in the future, each tenth newborn of the second comparison group had respiratory disorders (0% and 11.8%, p = 0.04). In the same group of newborns, signs of morpho-functional immaturity were diagnosed twice (4.1% and 7.8%, p = 0.711). After the anthropometric data were evaluated, all the newborns in the comparison group were diagnosed with IGR, but the newborns of the first comparison group exceeded all newborns of the second group in all anthropometric criteria: mean weight (2558.4 ± 26.2 and 2423.4 ± 32.1, p = 0.002), average height (49 ± 0,2 and 47,7 ± 0,2, p = 0,001), head circumference (32,1 ± 0,2 and 31,5 ± 0,2, p = 0,023), chest circumference 31.3 ± 0.2 and 30.2 ± 0.3, p = 0.003), indicating a later development of placental dysfunction in this group. The majority of newborns had a hypotrophic type of IGR, significantly more often in the first group (87.8% and 70.6%, p = 0.063), usually in the form of hypotrophy of I degree (77.6% and 54.9%, p = 0.029). Hypoplastic variant of IGR, on the contrary, was registered 2.4 times more often in newborns of the second group (12.2% and 29.4%, p = 0.063).

During the adaptation period (the first 3 days of life), only 8.2% of the newborns of the first comparison group had clinical signs of perinatal CNS disorders of the first comparison group; all newborns of the second comparison group had moderate or severe CNS perinatal disorders (p = 0.001). The most frequent clinical manifestations of perinatal CNS disorders were muscular hypotension (6.1% and 29.4%, p = 0.006), neuro-reflex excitation (2% and 21.6%, p = 0.007) and cerebral ischemia, which occurred only in three newborns of the second group (0% and 5.9%, p = 0.255). By the neurosonoscopy, subependimal cysts significantly more occurred in the newborns of the second group (2% and 15.7%, p = 0.042).

Thus, full-term low-weight newborns present different contingents not only in terms of severity and type of hypotrophy, but also in the initial state of the central nervous system. Considering that the degree of hypotrophy in full-term newborns is usually not highly expressed (late realization of placental dysfunction), they have a high frequency of late and unsatisfactory diagnosis of the intrauterine state of the fetus, post-factum diagnosis, which in the number of fetuses leads to marked changes in the central nervous system of hypoxic-ischemic origin. Prognostically unfavorable factors for the health of newborns are: the increased weight of the mother before the gestation period and thrombocytopenia preceding the childbirth. Pregnant women with the presence of markers of placental dysfunction according to Dopplerometry data, in the third trimester of pregnanc, are a risk group not only for IGR, but for perinatal CNS disorders. All full-term newborns born with low body weight require complex early diagnosis of markers of hypoxic-ischemic encephalopathy.

References

1. *The number and migration of the population of the Russian Federation in 2014*. Bulletin of the Federal State Statistics Service. 2015.

2. Fadeyeva N.I., Remneva O.V., Yavorskaya S.D. *Placental insufficiency: prevention, diagnosis, approaches to delivery, perinatal outcomes.* Barnaul. 2011.

3. Makarov I.O., Yudina Ye.V., Borovkova Ye.I. *Retarded fetal growth. Medical tactics: Training*. 3rd ed. Moscow: MEDpress-Inform; 2016.

4. Kady S.M., Gardosi J. Perinatal mortality and fetal growth restriction. *Best Pract Res Clin Obstet Gynecol*. 2004; 18: 397–410.

5. Pallotto E.K., Kilbride H.W. Perinatal outcome and later implications of intrauterine growth restriction. *Clin Obstet Gynecol*. 2006; 49: 257–69.

6. Y. Leitner et. al. Neurodevelopmental outcome of children with intrauterine growth retardation: a longitudinal, 10 year prospective study. *J Child Neurol.* 2007; 22: 580–7.

7.Barker D.J. Adult consequences of fetal growth restriction. *Clin Obstet Gynecol*. 2006; 49: 270–83.

8.Varvarigou A.A. Intrauterine growth restriction as a potential risk factor for disease onset in adulthood. *J Pediatr Endocrinol Metab*. 2010; 23: 215–24.

9. Malevich Yu. K., Shostak V.A. *Fetoplacental insufficiency*. Minsk: Belarus; 2007.

10. Order of the Ministry of Health of Russia from 01.11.2012 No. 572n (Edited on January 17, 2014) "On the approval of the order of medical care for the profile of obstetrics and gynecology (excluding the use of assisted reproductive technologies)" (Registered in the Ministry of Justice of Russia on 02/04/2013 No. 27960).

11. Dementyeva G.M. *Clinico-pathogenetic characteristics and criteria for the diagnosis of delayed growth and development of newborns*. [synopsis of thesis]. Moscow, 1984.

12. Glants S. *Medico-biological statistics*. Trans. from English. Moscow, 1998

13. Cody F. et. al. The effect of maternal obesity on sonographic fetal weight estimation and perinatal outcome in pregnancies complicated by fetal growth restriction. *Journal of Clinical Ultrasound*. 2016; 44 (1): 34-9.

Contacts

Corresponding author: Dolgova Nadezhda Sergeyevna – post-graduate student of the Department of obstetrics and gynecology with the course of FVE of ASMU, Barnaul.
Tel.: (3852) 36-85-87.
E-mail: author-dolgovans@yandex.ru

UDC 618.14-002:615.32

ENDOMETRIUM RECEPTION AND SOME IMMUNO-HISTOCHEMICAL PARAMETERS BY CHRONIC ENDOMETRITIS ON THE BACKGROUND OF TREATMENT BY THE EXTRACT OF ORTILIA SECUNDA

Altai State Medical University, Barnaul

O.A. Skoropatskaya, N.I. Fadeyeva, S.D. Yavorskaya, T.S. Taranina

Monocentric, prospective study in the group of female patients (p=20) with chronic endometritis, who received the extract of ortilia secunda at the stage of rehabilitation 2,5 mg/day during two menstrual periods. According to the therapy results there was stated a significant increase of estriol in the blood serum, activation of humoral antiviral immunity, restoration of endometrium structure and receptor apparatus of endometrium to sex steroids. The obtained results correspond to the results of the earlier experiment.
Key words: *ortilia secunda, chronic endometritis, phytotherapy, rehabilitation stage.*

Chronic endometritis remains an actual problem of modern gynecology, as frequently, after etiotropic therapy, there remain disorders of microcirculation in the endometrium, a decrease in the sensitivity of reception to steroid hormones, inadequacy of local immunity [1]. Different methods of pregravid preparation by chronic endometritis are suggested, aimed at eliminating the above-listed disorders. Most often used means are nonspecific immunomodulators, hormone therapy, a number of preformed factors [2].

The anti-inflammatory, anticoagulant and immunomodulating properties of the dry extract of ortilia secunda have been experimentally proven [3]. There was also proved the antimicrobial activity of decoction of the ortilia secunda leaves against enterobacteria, pseudomonads, staphylococcus, fungi of the genus Candida [4]. There had been previously conducted an experiment in 90 white outbred rats, which confirmed the efficacy of the ortilia secunda extract in the rapid restoration of the normal structure of the endometrium after experimental endometritis [5]. In a histological study of the endometrium, secretory changes were detected in rats which, for the purpose of treating experimental endometritis, received the ortilia extract. In the intact rats, such changes were not established. The data obtained suggested that the ortilia secunda extract possesses not only anti-inflammatory properties, but also contributes to the normalization of the endometrial receptor apparatus. The results obtained in the experiment were the reason for further research - the evaluation of the effectiveness of oral application of the ortilia secunda extract in patients with chronic endometritis.

Materials and methods

The research was conducted in accordance with the Helsinki Declaration of the World Association "Ethical principles of scientific medical research with human participation", as amended in 2000 and the "Rules of Clinical Practice in the Russian Federation" approved by the Order of the Ministry of Health of the Russian Federation of June 19, 2003 No. 266.

The study included 20 patients aged 30-35 years, with echoscopically and histologically verified chronic endometritis. The inclusion criteria were: verified chronic endometritis, as an indication for pregravid preparation. The exclusion criteria were other organic diseases of the genitals, anovulations, subcompensated and decompensated somatic diseases; the patient's age over 35 years. All patients in the rehabilitation phase after etiotropic treatment of endometritis were assigned the ortilia extract at the dose of 2.5 mg/day during two menstrual cycles. The ortilia secunda extract used in the experiment was obtained on the basis of the laboratory for study and use of natural resources of the Research-and-development Center of Fundamental medicine of the Altai State Medical University by means of vacuum-pulse extraction. This extraction technique makes it possible to obtain highly purified and highly concentrated grass extracts [6].

In the blood serum of all patients, before and after the intake of the ortilia extract, on the 21st-25th day of the menstrual cycle, along with standard tests (clinical and biochemical blood analysis, urinalysis, bacterioscopy and PCR diagnostics of the cervical canal) there were determined estradiol, estriol, progesterone, DHEA, testosterone, 17-OHP and cortisol, there was performed the evaluation of the interferon status of blood (alpha and gamma-interferons) [7]. Pipel-biopsy of the endometrium was carried out on the 5th-7th day of the menstrual cycle with subsequent histological and histochemical studies of the biopsy specimen. Immunohistochemical definition of the nature of inflammation was carried out according to the method of Ye.A. Mikhnina and co-authors

[8]. According to the proposed method, the number of cells of CD 56 +, CD 16+, HLA DR (II) + from 0 to 10 in the field of view is characteristic of healthy people. By the increase of only CD 56+ (above 10), autoimmune chronic endometritis is diagnosed.

By the increase in only CD 16+ and HLA-DR (II) + (above 10), chronic endometritis with exacerbation or acute endometritis is diagnosed. By the increase in all indicators, chronic endometritis is diagnosed, without exacerbation. Immunohistochemical (IHC) study of hormonal receptors of the endometrium (α-estrogen - ER and progesterone - PR) was carried out using monoclonal antibodies to α-ER (clone SP1, dilution 1: 200, Epitomics, USA), PR (clone YR85, dilution 1: 350, Epitomics, USA). The intensity of the IHC reaction of ER and PR was assessed by the method of the histological score "quickscore": A × B, where A is the percentage of positively stained cells (counting no less than 1000 cells in 10 fields of vision) [9].

Statistical analysis of the obtained results was carried out with the help of the program for statistical processing Statistic 10. The values of p were considered significant values by less than 0.05. The Shapiro-Wilk criterion was used to estimate the type of distribution of features in the case of small samples, in the case of large samples, the Kolmogorov-Smirnov test was used. The values of the interval values subject to the normal distribution are represented in the form M ± m, where M is the sample mean and the m - standard error of the mean. The values of qualitative characteristics are presented in the form of observed frequencies and percentages. In cases of normal distribution, as well as the equality of sample variances, Student's t-test was used to compare the samples.

Results and discussion

By the assessment of the hormonal profile of patients with chronic endometritis, it was established that the levels of all the hormones studied before and after treatment with the ortilia secunda extract were within the age limits. However, after the course of therapy, there was a significant increase in the average levels of estradiol, there was a trend towards an increase in the average level of estriol, progesterone and cortisol (Table 1), which can be regarded as an increase in ovarian activity under the influence of the ortilia seconda extract therapy.

Table 1

The dynamics of the average levels of certain hormones in the blood, taken on the 21st-23rd day of the menstrual cycle in patients with chronic endometritis

	Level of hormones before treatment	Level of hormones after treatment	P	Reference values on the 21st-23rd day of the menstrual cycle
Estradiol, pg / ml	152,78±7,34	209,22±10,43	p =0,004	48,3 -211
Cortisol, μg / dL	2,79±0,53	3,80±0,26	p =0,25	6,2-19,9
Estriol, nmol / l	0,8±0,35	1,26±0,31	p =0,004	0 - 1,4
Progesterone, nmol / l	5,47±2,62	7,01± 1,89	p =0,17	0,5-9,4
17-OHP, nmol / l	5,96 ± 0,32	5,67±0,33	p =0,98	1,0 – 11,5
ACTH, pg / ml	38,44±22,8	37,66±21,29	p =0,94	0 - 46
DHEA, μg / dL	295,5± 26,3	302,2±24,8	p =0,95	98,8-340
Total testosterone, nmol / l	0,32±0,03	0,36±0,02	p =0,99	0,45 - 3,75

The histochemical examination of the endometrium prior to therapy revealed an increase in CD20 +, CD138 +, natural killers (CD56 +, CD16 +) and lymphocytes expressing the activation marker of the histocompatibility complex of HLA-DR + II class, which is characteristic of chronic endometritis, without exacerbation [4]. A high level of CD56 + lymphocytes (from 25 to 60), detected in 30% of cases - a marker of pronounced autoimmune character of chronic endometritis [4].

After the course of therapy, there was a decrease in the frequency of detection in the endometrium of increased leukocyte activity: CD 20 + (100% and 15%, p = 0.0001); CD138 + (100% and 30%, p> 0.0002); CD 56+ (100% and 20% p> 0.0015); CD 16+ (100% and 20% p> 0.0015); HLA-DRII + (100% and 30%, p> 0.0002) in combination with a decrease in the average level of activated leukocytes: CD 20+ (11.15 ± 1.2 and 3.0 ± 1.2 p = 0.0002); CD138 + (16.44 ± 3.4 and 1.55 ± 0.2, p> 0.0025; CD 56+ (26.88 ± 3.6 and 9.55 ± 0.32, p = 0.015); CD 16+ (17.3 ± 2.43 and 9.11 ± 0.18, p = 0.009), HLA-DRII + (19.55 ± 4.23, 9.88 ± 2.3, p = 0.006).

Immunohistochemical (IHC) study of hormonal receptors of the endometrium (α-estrogen - ER and progesterone - PR) in dynamics allowed us to establish that upon completion of therapy with the orthilia secunda extract in all patients, the average level of estrogen receptivity of the endometrium increased significantly, as in stromal cells (22,22 ± 2.3 and 44.88 ± 2.6 p = 0.00003) and in the glands (20.22 ± 4.2 and 41 ± 2.2, p = 0.00003). In addition,

the mean progesterone receptivity of the endometrium in the stromal cells (32.55 ± 4.3 and 48.77 ± 4.3, p = 0.008) and in the glands (32.66 ± 2.2 and 48.44 ± 2.2, p = 0.02) significantly increased.

By the evaluation of the level of alpha and gamma interferons in heparinized blood on 21st-23rd day of the cycle, the initial insufficiency of the first degree of alpha interferon was detected in 20% of cases, whereas at the completion of therapy, the frequency of its detection increased to 40% of cases (p = 0.08), which was accompanied by a decrease in the average level of the studied indices in the blood (849.55 ± 120 and 646 ± 120 U/ml, p = 0.06).

Lack of gamma interferon of the first degree was initially determined in 90% of cases, which may be associated with insufficient antiviral immunity [1]. Upon the completion of therapy, insufficiency was detected only in 10% of cases (p = 0.0001), there was a general tendency to the increase of the average level of gamma interferons (104.44 ± 6 and 193.55 ± 4 U / ml, p = 0.0001), which can be regarded as the restoration of antiviral and antiproliferative activity [1,2].

By the histological examination of the endometrial pile-biopsy specimens, on the 5th-7th day of the cycle, inflammatory infiltrates were detected in all cases: diffuse infiltrates in 40% and focal infiltrates in 60% of cases. Upon completion of therapy, all patients showed an improvement in the histological pattern in terms of a decrease in the frequency of leukocyte infiltration, represented only by the diffuse form (100% and 20%, p = 0.00001). Focal fibrosis, revealed initially in 50% of cases, was preserved after the therapy only in 20% of cases, in combination with diffuse leukocyte infiltrate. In each second patient, after the completion of the course of therapy, histological markers of chronic endometritis is not established.

Thus, a two-month course of chronic endometritis therapy with the ortilia secunda extract at the dose of 2.5 mg/day significantly increased the average level of estradiol in the blood serum and the average level of estrogen and progesterone receptors in the endometrium; activated humoral antiviral immunity by reducing the frequency of gamma-interferon deficiency and increasing the average level of gamma interferons in the blood; increased the frequency of the normal value of activated leukocytes CD 20+, CD138 +, CD 56+, CD 16+, HLA-DRII +; eliminated focal leukocyte infiltrates and reduced diffuse leukocyte infiltrates while maintaining focal fibrosis.

References:

1. W. Amir, B. Micha, H. Ariel, L.G. Liat, D. Jehoshua, S. Adrian Predicting factors for endometrial thickness during treatment with assisted reproductive technology. *Fertil Steril*. 2007; 87(4): 799–804.

2. Lomboyeva S.S., Olennikov D.N., Tankhayeva L.M. Pharmacognostic study of the aboveground part of Ortilia Secunda (Ortilia secunda (L.) House). *Chemistry of plant raw material.* 2010; 109-114.

3. D. D. Carson, I. Bagchi, S.K. Dey, A. C. Enders, A. T. Fazleabas, B. A. Lessey, K. Yoshinaga Carson, D. D. Embryo implantation. *Developmental biology*. 2000; 223 (2): 217– 237.

4. B. Feinen, A. E. Jerse, S. L. Gaffen, M. W. Russell Critical role of Th17 responses in a murine model of Neisseria gonorrhoeae genital infection. *Mucosal immunology*. 2010; 3 (3): 312– 321.

5. Iwasaki, A. Antiviral immune responses in the genital tract: clues for vaccines. *Nature reviews. Immunology*. 2010; 10(10): 699– 711.

6. Janeway, C. A. The immune system evolved to discriminate infectious nonself from noninfectious self. *Immunology today*. 1992; 13 (1): 11– 16.

7. Lieberman, J. The ABCs of granule– mediated cytotoxicity: new weapons in the arsenal. *Nature reviews. Immunology*. 2003; 3(5): 361– 370.

8. Mikhnina Ye.A., Davydova N.I., Kalinina N.M., Elinidi V.N. Hormonal and immune changes in formation of endometrium pathology in women witexternal genital endometriosis. *Journal of obstetrics and woman disease.* 2006; 4)4): 1-14.

9. R.G. Lopes, E.C. Baracat, L.C. de Albuquerque Neto, J.F. Ramos, S. Yatabe, D.B. Depesr, U.G. Lippi, J. Minim. Analysis of estrogen– and progesterone–receptor expression in endometrial polyps. *Invasive Gynecol*. 2007; 14(3): 300–303.

10. G. Mor, I. Cardenas. The immune system in pregnancy: a unique complexity. *American journal of reproductive immunology*. 2010; 63(6): 425– 433.

Contacts

Corresponding author: Skoropatskaya Olga Alekseyevna, post-graduate student od the Department of Obstetrics and Gynecologyof Altai State Medical University, Barnaul. 656038, Barnaul, ul. Molodezhnaya, 9.
Tel.: 8-906-942-9138.
Email: lipsi-gutan@yandex.ru

UDC 618.256-005.2

ALTERATIONS OF THE HEMOSTATIC SYSTEM IN THE IVF CYCLE AND THEIR INFLUENCE ON THE PROCEDURE EFFICIENCY

[1]Altai State Medical University, Barnaul
[2]Scientific center of hematology of the Ministry of Health of the Russian Federation, Barnaul
[3]The Ministry of Health of Altai Krai, Barnaul
[4]Altai regional clinical perinatal center, Barnaul

A.P.Momot[1,2], I.V. Molchanova[1,2,3,4], L.P. Tsyvkina[1]

In vitro fertilization (IVF) is widely used for the solvation of infertility problem. Its failures are connected with a number of reasons including disorders of the hemostatic system. In terms of the current study, there were analyzed risk factors of IVF failures before and after the therapeutic correction of excessive generation of thrombin and hypofibrinolysis.

Key words: *extracorporal fertilization, pregnancy, thrombin generation, hypofibrinolysis, heparin prevention, vasocompression.*

Today, assisted reproductive technologies (ART) and, first of all, in vitro fertilization (IVF) and embryo transfer (ET), intracytoplasmic sperm injection (ICSI), oocyte donation (OD) have turned from unique techniques for our country to almost routine ones, performed in terms of the program compulsory medical insurance (CMI).

The technique of these procedures is worked out in great detail and is successfully applied in ART centers throughout the country. In 2010, according to the data of the Ministry of Health of the Russian Federation in 2015, 37 664 cycles of ART in the framework of the CMI program were conducted in our country, which is 12 137 cycles more than in 2012. The wide introduction of ART, a sufficiently high level of qualification of reproductive and embryology specialists allowed not only to increase the number of cycles conducted throughout the country, but also to increase their efficiency to 32-33% [3, 4].

However, it should be recognized that an increase in the number of cycles and the accumulation of medical experience led to an avalanche-like increase in information about the problems of ART, including in regional centers, which include the Regional Center for the Preservation and Rehabilitation of Reproductive Function in Barnaul. The Center was established in 2009 with the support of the Governor of Altai Krai A.B. Carlin. The Center made a high-tech way for overcoming infertility available to the population, since its funding was initially provided at the expense of the regional budget. The state form of the work of this center allowed to accumulate invaluable experience and in the future to switch over to work on compulsory medical insurance, having increased volumes 4 times from 300 to 1200 cycles per year. At the same time, despite the annual increase in the effectiveness of ART, this issue is still acute.

It is known that pregnancy largely depends on two components - a functionally complete embryo at the blastocyst stage and endometrial receptivity, which, according to modern concepts, are crucial in achieving optimal implantation conditions, and that many of the mechanisms involved in this process (hormonal interactions, pinopodia formation, local immunity, autoimmune reactions, a complex of molecular and cellular interactions regulated by para- and autocrine factors, the formation of an implantation window), have not yet been studied sufficiently deeply [1, 2, 8, 9, 13.]. It is logical to assume that implantation is the most vulnerable stage of the IVF / ICSI and ET program both from the point of view of pregnancy and its bearing in the early stages. Undoubtedly, in all cases of absence or inferior implantation with further loss of pregnancy, the combined effect of various adverse factors is important. It may sound paradoxical, but the factors affecting the implantation processes and promote early pregnancy loss after IVF are considered by most authors to be the use of drugs - inductors of superovulation, which create prerequisites for abortion along with a high level of follicle-stimulating hormone, a change in the hormonal background, the phenomenon of hypercoagulation as a result of activities aimed at the maturation of follicles [5, 6, 7].

Among the persistent scientific searches for the role of thrombophilia in the formation of obstetric-gynecological pathology, there is no doubt that the stimulation of superovulation in the cycle of IVF is a factor of thrombogenic risk, contributing to the development of hypercoagulable syndrome, which is laboratory confirmed by an increase in von Willebrand factor, V and VIII factors, fibrinogen, Resistance of APC- (activated protein C), against the background of a decrease in the activity of the main physiological anticoagulants - antithrombin, proteins C and S. [10, 11, 12.]. However, scientific studies that determine the influence

of these haemosteziologic changes on the receptive properties of the endometrium and, ultimately, on the outcomes of in vitro fertilization are very few. But even in these works, diagnostic markers are not presented with clear, clinically relevant parameters of shifts in the hemostatic system, which are so important for the practice of the doctors of reproductive centers. Therefore, up to now, the issue of correction of changes in the system of hemostasis that have arisen against the background of stimulation of superovulation remains open and controversial, which served as a motive for our own research conducted by the Altai Branch of the Hematology Research Center of the Russian Ministry of Health and the Altai State Medical University.

Materials and methods

There were studied the changes in the coagulation hemostasis and fibrinolytic properties of 163 patients with tubal peritoneal infertility, which were exposed to IVF. Inclusion parameters:

1. Tubal-peritoneal factor of infertility
2. Age under 35 years
3. 1-2 unsuccessful IVF attempts in medical history
4. Identical protocol for the stimulation of superovulation (long-term protocol with the use of diperelin (0.1 mg) or decapeptil (0.1) and gonadotropins (puregon 150-250 IU or 225 IU). All patients received progesterone (dufaston) from the moment of follicular puncture at the dose of 30 mg per day, from the moment of embryo transfer - 40 mg per day.
5. Good quality embryos (Class A and B)

Exclusion parameters**:**

1. Pathology of the reproductive system that changes the receptive properties of the endometrium (congenital malformations, tumor and hyperplastic processes, adenomyosis)
2. Severe somatic pathology (systemic, endocrine, autoimmune diseases)
3. Decreased ovarian reserve
4. Co-factor of infertility (male, endocrine)

The patients were young, without any special health problems, both reproductive and somatic, and each of them had an unsuccessful attempt at IVF in the anamnesis.

By the choice of methods for the study of hemostasis, there were used modern, universally recognized and widely used markers of thrombotic readiness, reflecting the state of both coagulation hemostasis and fibrinolytic activity. However, it was taken into account that different factors of thrombogenic risk lead to violations at various stages of the coagulation cascade and fibrinolysis, and this process is accompanied by the appearance of markers specific for this vector of activation of the hemostasis system, which often makes interpretation of these data difficult.

This circumstance served as a motivation for the use of fundamentally new integral tests in the study, that allow to evaluate the final stage of blood coagulation. These tests include thrombin generation test (TGT) - thrombin peak concentration nmol/l and endogenous thrombin potential (ETP) (nmol x min). Its versatility lies in the fact that it reflects various mechanisms of activation of hemostasis, both coagulative and cellular, which together inevitably lead to thrombinemia.

Fibrinolytic blood activity was assessed using the index of fibrinolysis-activating capacity of the endothelium (FACE index,%), which was calculated from the ratio of the activity of the tissue activator plasminogen and its inhibitor, the lysis time of the clot.

The study of hemostasis was carried out three times, before the stimulation of superovulation, before puncture of the follicles and on the day of HCG.

Results and discussion

As a result of the study, it was found that changes in hemostasis against the background of stimulation of superovulation were detected in 114 patients (70%), low fibrinolytic activity before entry into the cycle - in 36 patients (22%), signs of hypercoagulation - in 78 patients (48%)

In this case, the data given in Table 1 show, that stimulation of superovulation causes an increase in thrombin generation and practically does not affect the parameters of fibrinolytic activity of the plasma. The current study data fully agree with the findings published in 2012 by Westerlund, who believes that fibrinolytic activity has individual characteristics in each case [14].

When carrying out a correlation analysis of the detected changes in the hemostasis system and the unsuccessful attempts of IVF, it was determined that a high correlation was found between excessive generation of thrombin and low fibrinolytic activity of the vascular wall with unsuccessful outcomes of IVF. The correlation coefficient (according to Spearman) was 0.88 ($P < 0.002$) and 0.67 ($P < 0.02$), respectively (Table 2).

Further, there were determined the threshold values of these tests, related to the failures of IVF. It turned out that pregnancy does not occur with an increase in endogenous thrombin potential against the background of stimulation of superovulation over 1900 nmol x min and PCC over 360 nmol x l (Table 3).

Failures of IVF are observed in patients with reduced fibrinolytic activity of the vascular wall according to the calculated FACE index less than 11% (Table 3).

Based on the obtained objective laboratory criteria, there were formed the groups of women who need therapeutic correction of hemocoagulation.

In patients with high thrombin generation there were used low molecular weight heparins, in women with impaired fibrinolysis, vasocompression therapy was used. Combined therapy was used by the combination of these changes. In the center for the preservation and restoration of the reproductive function, these therapies were applied in 98 women entering the IVF cycle, and the outcomes of assisted reproductive technologies were compared with a group of 154 women with identical changes in hemostasis not receiving the proposed therapy.

In the course of the study, it was determined that the use of low molecular weight heparin, starting the day after follicle puncture, led to a regular decrease in the intensity of the thrombin generation test, similar to the peak concentration of thrombin.

Similar results were obtained in patients with combined therapy with heparin and vasocompression. In women who underwent such therapy, the parameters reflecting both the rate of fibrin formation and the fibrinolytic activity of blood plasma and the vascular wall were normalized.

As a result, it has been established that all types of treatment undertaken by us reduce the risk of a negative outcome of IVF. Reduction of relative risk (RRR) with the use of heparin therapy was more than 25%, which corresponds to a clear clinical effect. In particular, the reasonable designation of low molecular weight heparin with high thrombin generation helps to reduce the number of unfavorable outcomes of in vitro fertilization by 33%. When combining prophylactic doses of low-molecular heparin with vasocompression in the case of combined hemostasis and fibrinolysis, the relative risk was more than 50%, indicating a pronounced clinical effect. In this clinical group there was a decrease in the number of failures in the cycle of IVF by almost 40%.

Table 1

Dynamics of hemostasis and fibrinolysis (M + SD) by superovulation and embryo transfer to the uterine cavity (n = 163)

Index	1 observation point	2 observation point	3 observation point
ETP, nmol/min	1461,2±81,6 P_{1-2}<0,001	1849,3±89,2 P_{2-3}<0,001	1891,4±53,5 P_{1-3}<0,001
PCC, nmol/l	310,1±23,1 P_{1-2}<0,001	382,4±19,5 P_{2-3}<0,001	375,6±25,2** P_{1-3}<0,001
t-PA, activity, un/ml	0,30±0,16 P_{1-2}>0,5	0,33±0,15 P_{2-3}>0,5	0,31±0,15 P_{1-3}>0,5
PAI-1, activity, un/ml	2,40±1,13 P_{1-2}>0,5	2,50±1,22 P_{2-3}>0,5	2,44±1,98 P_{1-3}>0,5
FACE index, %	12,5±3,1 P_{1-2}>0,5	13,2±4,2 P_{2-3}>0,5	12,7±3,6 P_{1-3}>0,5
Clot lysis time, min	9,4±3,0 P_{1-2}>0,5	8,7±3,3 P_{2-3}>0,5	10,1±3,5 P_{1-3}>0,5

Note: * - in this table, the significance of differences compared to the control indicators - P_c <0.05; ** - the same, P_c <0.01.

Table 2

Estimation of informative value of changes in hemostasis parameters to unsuccessful outcomes of IVF

Index	Correlation coefficient (P – significance)	
	at the 1st point of observation	at the 2nd point of observation
ETP, nmol/min	0,03 (0,26)	0,79 (0,001)
PCC, nmol/l	0,08 (0,44)	0,88 (0,002)
Level of plasminogen, %	- 0,31 (0,23)	- 0,24 (0,31)
Clot lysis time, min	0,71 (0,01)	0,66 (0,02)
t-PA, activity, un/ml	0,60 (0,02)	0,52 (0,01)
PAI-1, activity, un/ml	0,51 (0,01)	0,47 (0,02)
FACE index, %	0,67 (0,02)	0,53 (0,01)

Table 3

Factors contributing to the failure of pregnancy in the cycle of IVF (n = 163)

Feature	IVF failure (n=107)		IVF success (n=56)		MIS (0,95% CI*)	P
	Abs	%	Abs	%		
2. ETP over 1900 nmol / min (at the 2nd point of observation)	73	68,2	4	7,1	27,9 (9,33-83,4)	<0,00001
3. PCC over 360 nmol / l (at the 2nd point of observation)	74	69,1	5	8,9	22,8 (8,36-62,5)	<0,00001
5. Clot lysis time over 12 min (at the 1st point of observation)	69	64,5	5	8,9	18,5 (6,81-50,3)	<0,000001
6. FACE index under 11% (at the 1st point of observation)	62	57,9	6	10,7	11,5 (4,53-29,1)	**0,00005**
9. FII gene mutation (G/A, A/A)	3	2,8	0	0	3,78 (0,19-74,5)	0,319
11. Endometrial Hypoplasia	11	10,3	3	5,3	2,02 (0,54-7,57)	0,383
12. IVF failure in anamnesis	20	18,7	6	10,7	1,91 (0,72-5,08)	0,260
13. Homocysteine in the blood over 15 mkmol / l (at the 1st point of observation)	22	20,5	7	12,5	1,81 (0,72-4,54)	0,280
16. ombination of polymorphisms MTHFR (G/A, A/A) и PAI-I (G/A, A/A)	26	24,3	9	16,0	1,67 (0,72-3,87)	0,315
18. PAI-I gene polymorphism (G/A, A/A)	42	39,2	20	35,7	1,16 (0,59-2,27)	0,735
20. Hyperfibrinogenemia over 5.0 g / l (at the 2nd point of observation)	14	13,0	7	12,5	1,05 (0,39-2,78)	>1,00
21. The level of D-dimers over 500 ng / ml на (at the 2nd point of observation)	19	17,7	10	17,8	0,99 (0,42-2,31)	>1,00
26. MTHFR gene polymorphism (G/A, A/A)	30	28,0	23	41,0	0,55 (0,28-1,10)	0,113
27. FV Leiden gene mutation (G/A)	1	0,9	1	1,7	0,51 (0,03-8,45)	>1,00

Table 4

Influence of used methods of correction of hemostatic and fibrinolytic reactions on the efficacy of IVF

Means of therapeutic effect	Those in need of treatment, but not treated (subgroups 1.2 and 2.2.4, n = 154)			Those in need of treatment and treated (subgroups 2.2.1, 2.2.2 and 2.2.3, n = 98)		
	Abs.	Pregnant	%	Abs.	Pregnant	%
Heparin prophylaxis	64	4	6,2	38	15	39,5
PPK course	48	7	14,6	23	10	43,5
Combined effect	42	3	7,1	37	17	45,9

Table 5

Effectiveness of various therapies in women in the IVF cycle

Means of therapeutic effect	Index					
	ARR	NNT	OR	CI 95% (RRR)	CI 95% (OR)	RRR%
Heparin prophylaxis	0,27	3,7	0,22	0,52-0,92	0,07-0,69	31
PPK course	0,31	3,2	0,19	0,44-0,94	0,05-0,65	35
Heparin prophylaxis combined with PPK course	0,55	1,8	0,24	0,23-0,60	0,02-0,22	63

References

1. Agadzhanova L. Endometrial Pinopodia as Markers of Human Implantation. *Probl. reproduct*. 2004; 3: 6–11.
2. Burleyev V.A., Kuzmichev L.N., Shchetinina N.S., et al. The state of the molecular implantation window: the role in the outcomes of IVF (literature review). *Probl. reproduct*. 2009; 6: 24–27.
3. Vartanyan Ye.V., Aizikovich I.V., Antonov A.R. Causes of IVF failures. (literature review). *Probl. reproduct*. 2010; 3: 57-61.
4. Korsak V.S., ART in Russia. Report for 2007. *Probl. reproduct*. 2009; 6: 14–15.
5. Makarov O.V., Kerchelaeva S.B., Ozolina L.A. *Acquired and hereditary factors of thrombophilia in the development of complications of pregnancy*. Moscow, 2006.
6. Makatsariya A.D., Dolgushina N.V. *Herpetic infection. Antiphospholipid syndrome and fetal loss syndrome.* Moscow: Triada-X, 2008.
7. Pilipenko M.A. *The significance of thrombophilia in the formation of early embryonic losses during in vitro fertilization and embryo transfer*. [synopsis of thesis]. Omsk, 2009.
8. Poletayev A.B. *Immunophysiology and immunopathology (selected chapters)*. Moscow: LLC "Medical Information Agency", 2008.
9. Radzinsky V.Ye., Orazmuradov A.A. ed., *Early pregnancy (2nd ed., rev. and add.).* Moscow .: Status Praesens, 2009.
10. Rudakova Ye.B., Loboda O.A., Poltoraka Ye.V., et al. Pathology of hemostasis and chronic endomyometritis as a cause of failure and embryonic loss in in vitro fertilization. *Journal of Ural Medical Academic Science*. 2008. 2 (20): 59–60.
11. Rudakova Ye.B., Poltoraka Ye.V., Luzin A.A., et al. Possibilities of increasing the effectiveness of assisted reproductive technologies. *Bulletin of NSU.* 2009; 7(2): 20–24.
12. Rudakova Ye.B., Poltoraka Ye.V., Pilipenko M.A., Loboda O.A .Role of chronic endometritis and thrombophilia in the formation of preembryonic and embryonic losses. *Col. of theses of the All-Russian scientific-practical conference "Outpatient and polyclinic practice - the platform of women's health"*. Moscow, 2009. 1: 33–34.
13. Svetlakov A.V., Yamakova M.V., Yegorova A.B., Mikutkina S.V. Molecular-biological aspects of implantation in humans and animals. *Probl. reproduct*. 2002; 2: 16–28.
14. *Westerlund E., Henriksson P., Wallén H., Hovatta O., Rodriguez-Wallberg K., Antovic A.* Detection of a procoagulable state during controlled ovarian hyperstimulation for in vitro fertilization with global assays of haemostasis. *Thromb. Res*. 2012; 46(4): 417-425.

Contacts

Corresponding author: Momot Andrei Pavlovich, Doctor of Medical Sciences, Professor, Head of the laboratory of hematology of Central research and development laboratory of ASMU, Barnaul.
656045, Barnaul, ul. Lyapidevskogo, 1.
Tel.: (3852) 689-800
Email: xyzan@yandex.ru

REQUIREMENTS FOR PUBLICATION IN THE «BULLETIN OF MEDICAL SCIENCE» JOURNAL

Journal "Bulletin of Medical Science" publishes original researches, case reports, scientific reviews, discussions, sponsored articles and advertisements. All journal sections focus on medical subjects.

The following requirements for publication in the «Bulletin of Medical Science» Journal were developed according to the uniform requirements, stated by the International Committee of Medical Journal Editors (ICMJE) in the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publication".

MAIN REQUIREMENTS:

1. The article must be followed by official referral of the organization where the work has been done, expert report and scientific supervisor's signature.

2. The article must be published on white paper sheets, A-4 size, on one side of the paper sheet, field width – 2,5-3 cm. 2 copies of the article should be sent to the editorial office.

3. Write initials and surnames of all the authors, the title of the article, the organization where the article has been written at the top of the first page. The signatures of all the authors are required at the end of the article. On a separate page there must be written full names of all authors of the article, and also the address, contact numbers, E-mail of one of the authors for the contact with editorial staff.

4. Article length – 12-18 thousand spaced characters. The number of pictures and tables in accordance with article length. The data presented in the tables must not duplicate the data of the figures and the text, and vice versa.

5. The article must include an abstract in the Russian and English languages. Each of them must be typed on a separate page. Abstract length – 0,5 of a page. At the beginning of an abstract there should be initials and surnames of all the authors and the title of the article. As a new paragraph write 3-5 key words at the end of an abstract.

6. The article must be well-edited by the author. The content of the article must be easy to understand, without long introductions and repetitions. International System of Units (SI) must be used. If you used the apparatus with other units, then all of them must be converted into SI system. Conversion factor or computer program used for the conversion must be mentioned in the section "Materials and methods".

7. Only generally accepted abbreviations are allowed. Firstly, the term must be fully mentioned, then abbreviated. Use only capital letters in abbreviations.

8. Special terms should be given in Russian transcription. Chemical formulas and doses are visaed by the author. Mathematic formulas must be prepared specialized mathematical computer programs or formula editors of "Equation" type.

8. The pictures must be clear, photos – contrasting. On the back of each illustration write the first and the last name of the first author, first two words from the title of the article, the number of the picture; mark the up and down of the picture by the words "up" and "down" in appropriate places. All this information must be written with an ordinary pencil without pressing. Picture captions must be given on a separate page together with the author's surname and the title of the article, the number of the picture, with the explanation of the meaning of all curved lines, letters, numbers and other symbolic representations.

10. The tables must be demonstrable, have the title, sequence number; the headings must be relevant to the content of columns. Each table should have a reference in the article.

11. The article with original research should have the following parts: 1. "Introduction", 2 "The Purpose of the Research", 3. "Materials and Methods"; 4 "Results"; 5. "Discussion", 6. "Conclusion". In the part "Materials and Methods" there should be given a detailed description of the methodology of the research, the equipment used in the research, the number and characteristics of patients. The principle for the dividing of patients into groups and the design of the research must be compulsory given. This part must contain comprehensive information for further reference to these results by other scientists, for comparing with the results of analogous works and for the possibility of including the data of the article into meta-analysis. At the end of the part "Materials and Methods" there should be a smaller part "Data Processing" . The full list of all used statistical methods of analysis and criteria of hypothesis testing must be given. It is not allowed to write "standard statistical methods were used" without their specific indications. It is compulsory to mention the accepted in the research the critical level of significance "p" (e.g. "The critical level of significance in case of statistical hypothesis testing in this research is 0,05"). In each specific case there must be given the actual value of the reached level of significance "p" for the used statistical criterion (not just "p<0,05" or "p>0,05"). Besides, it is necessary to state specific indications of the received statistical criteria (e.g. criterion "Chi-square" = 12,3 (number of degrees of freedom df = 2, p=0,0001). It is compulsory to give the definition for all used statistical terms, abbreviations and symbolic notations (e.g. M – sample mean, m (SEM) – error

in mean, STD – sampling standard deviation, p-reached level of significance). In case of combinations like M±m it is necessary to give the meaning of each symbol, and also sample volume (n). If the used statistical criteria have limitations in their usage, specify how these limitations were checked and what the results of these checks are (e.g. in case of using parametric methods it is necessary to show how the normality fact of sample distribution was proved). Avoid non-specific usage of terms which have a few meanings: (e.g. there are a few variants of correlation coefficient: Pearson, Spearman and others). Average quantities should not be given more precisely than for one decimal mark in comparison with base data, mean-square deviation and error in mean – for one more mark precisely.

12. The literature list must be typed on a separate page, each source from the new line with sequence number. The numeration must be done according to the order of citation of the source in the article. The author is responsible for the correctness of the literature list data. The names of foreign authors are given in authentic transcription.

13. The text should be duplicated in the electronic form in WORD (the text is typed without paragraph breaks, hyphenation) and be sent on a CD and (or) by e-mail with the note "For the Bulletin of Medical Science". Each picture\photo should be sent as a separate .jpeg file, resolution not less than 300 dpi. The tables and diagrams must be sent in EXCEL, the name of the file must be the same as the name of the basic file. The format of the file with the article should be compatible with MS Word.

14. The editorial board reserves the right to edit the sent articles. The reviews on the articles are sent to the authors upon written request.

15. The articles not following the stated requirements are not reviewed, the sent articles are not returned back.