SLEEPING SICKNESS IN ECOLOGICALLY UNFAVORABLE REGIONS OF KAZAKHSTAN

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A new, unknown to science sleeping sickness has appeared in Kazakhstan. It chronically overtakes dozens of residents of the village of Kalachi, Akmola region. The first official case occurred in March and April 2013, the last in August 2014. Locals between the ages of 14 and 70 go to the dispensary with the same symptoms - weakness, drowsiness, dizziness, impaired coordination of movements and partial loss of memory. They sleep up to 2 days. Studies conducted by doctors and environmentalists have not identified the causes of the disease. There is a question of evicting residents and declaring the village the place of the epidemic. The main reason for the lack of diagnosis is a weak material and methodological base, the absence of morphological changes and in-depth molecular biomedical research.

The scientific novelty of the project lies in the fact that for the first time at the molecular level a new, unknown to science sleeping sickness is being studied. The data obtained will reveal the composition of peptides and proteins, LMWOC, which are the main mediators of reactions and human behavior. These compounds will be identified and their concentration determined. The revealed differences in the composition of LMWOC, proteins and peptides, and enzymes will reveal the molecular markers responsible for the manifestation of narcolepsy. The practical significance of research is to identify the causes of sleeping sickness at the molecular level and to develop practical methods for its treatment. The purpose of the study is to study the molecular features of a new sleeping sickness localized only in the village of Kalachi in Kazakhstan, to identify molecular markers, to develop recommendations for eliminating the epidemic.

The goal is to conduct a study of environmental living conditions and biomedical parameters in sick residents of the village of Kalachi. Using proteomic analysis, gas and liquid chromatography to study the composition of proteins and peptides, low molecular weight organic compounds (LMWOC) in the blood and urine of patients in the process of sleep and recovery, to identify molecular markers of the disease. To study the component composition and activity of molybdic enzymes associated with the detoxification of xenobiotics. To study the metabolism of orexin or hypocretin associated with falling asleep during illness in patients during sleep and recovery. It is important to economically develop recommendations for the use of molecular markers in the treatment of sleeping sickness in the village of Kalachi.

Literature Review

In the current literature, cases of involuntary falling asleep of a person are known. They refer to encephalopathy - a generic name for non-inflammatory, in contrast to encephalitis, brain diseases. African trypanosomiasis can be ruled out immediately, since the disease lasts several years, is associated with skin diseases and brain damage. In our case, there are examples in the village of Kalachi, when a man suddenly fell asleep, who spent only 3 days in the village. There remain 3 other diseases and an assumption about the effect of radon on sudden falling asleep. It is known that gases, such as xenon, can cause anesthesia in humans. Radon as an inert gas can have the same ability and accumulate in the body. Encephalopathy may be associated with infectious diseases. In the case of Kazakhstan sleeping sickness, only some of the diseases were investigated;

no deep screening was carried out.

What is new in our work is that thousands of organic compounds that are not usually analyzed in medical practice will be analyzed. These compounds, in general, will create a picture of the patient's metabolism, i.e. metabolomics. It will be possible to establish the direction of changes in metabolism by these indicators and identify marker compounds of the disease. In addition, all proteins and peptides of patients will be analyzed in terms of composition in blood and other fluids. Based on the fact of complete decoding of the human genome, it will be possible to identify the direction of synthesis of new or previously existing proteins and peptides in the body. In addition, in the case of a new infectious disease, it will be possible to identify new proteins and identify them through libraries of genes and proteins of viruses and bacteria. This set of methods is universal, because similar changes in the studied compounds occur under various influences. In our case, it will be possible to identify either a new disease or a viral, bacterial disease. The main reason for the lack of diagnosis is a weak material and methodological base, the absence of morphological changes and deep molecular biomedical research.

Methodology

The objects of study are residents of the village of Kalachi with sleeping sickness and the environmental conditions of their residence. Patients will be taken blood and urine samples during sleep and in dynamics within a week after waking up. Samples will be analyzed for peptide and protein composition using proteomic analysis.

Mass-Spectrometer analysis. The resulting peptides were analyzed using nano-HPLC (Agilent Technologies 1200), which is directly connected to the ion-trap mass spectrometer (Bruker 6300 series), equipped with a source with a nano-electrospray. Separating acetonitrile gradient from 5% to 90%, duration 25 minutes. The fragmentation voltage is 1.3 V. The ion trap has sequential sets of 4 scanning modes, consisting of: fully scanning MS in the ranges above 200-2000 m / g, accompanied by three MS / MS dependent scanners of the three most common ions at full scanning. Protein identification will be performed by the Mill Spectrum software package. Quantitative analysis of the spectrum and chromatograms will be carried out by Data Analysis for the 6300 series Ion Trap LC / MS, software package version 3.4. The relative content of each peptide in different fractions was determined by comparing the peak areas with the total ion chromatogram area (TIC) for this peptide.

Studies of low molecular weight compounds with a molecular weight (LMWOC) up to 2000 will be carried out on an Agilent 6890 gas chromatograph, GCMS. Will be carried out: derivatization of components, modern libraries of organic compounds will be used for identification, numerous taps of the most common compounds will be used.

Qualitative and quantitative analysis of the chemical composition will be determined on a gas chromatograph-mass spectrograph GCMS-QP5000, Shimadzu, Agilent Agilent 6890N with a mass-selective detector: Agilent MSD 5973 and a liquid chromatograph from TSQ Vantage Triple Stage Quadrupole, Thermo Scientific based on the faculty of natural Sciences Eurasian National University. L.N. Gumilyov. For the identification of chemical compounds, the databases NISTO2.L, Wiley7n.1, PMW_Tox3.1 will be used.

Statistical processing of the results will be carried out using the software package "Statistica 6.0". Intergroup differences will be evaluated by the non-parametric Mann-WhitneyU-test. For paired groups, the nonparametric Wilcoxon test will be applied.

Table 1.

General statistical indicator of the population, people. (2011, 2018)

Population	Krasnogorsk village		Kalachi village	
	2011	2018 (9-months)	2011	2018 (9-m
Total	210	22	555	32(
Adults	192	17	451	28(
Children	13	2	90	26

Adolescents	5	3	14	11
Males	152	8	211	15
Females	58	14	240	13

Findings

In order to identify the general psychophysiological state, as well as other psychological features in the framework of the study of the problem of sleeping sickness in the city of Kalachi, the following methods of psychological research were carried out: questioning on the general psychophysiological state; Holmes and Rage stress test; Bass-Darki aggressiveness survey. Survey results showed that at the time of the survey, many respondents who experienced sleeping sickness from 1 to several times -88% noted a general deterioration in their health status, 35% of them noted that they noticed nervousness, 7% - they have heart problems, 28% complain of memory impairment, 7% complain of problems with the thyroid gland. The subjects - 14% also noted that they have high blood pressure, and regular headaches, which are reported by almost 94% of respondents. 93% of the subjects noted their general fatigue, of which 14% during physical exertion and 14% when the weather changed. To the question “Do they dream?” - 60% of the respondents answered in the affirmative, several of them rarely dream, and 24% of those surveyed do not dream at all during active sleep, in addition, 18% of them suffer from insomnia. There is also psychological fatigue against the general background of physical fatigue, 24% the subjects noted that they were experiencing stress, 14% of them said that periodically their arms and legs were wrung.

Stress resistance is an important feature of the psychological health of a person, indicating psychological health and well-being. Due to the fact that stress tolerance is the ability to withstand certain psychophysical stresses and tolerate stress without harming the body and psyche, the purpose of this test was to study the adaptive potential of the examined after they fell ill with sleeping sickness. In particular, the importance of the adaptive potential reveals the degree of hidden capabilities of the patient with "sleeping sickness" to optimally integrate into new or changing conditions of his social environment (in this case, the presence of the disease). In general, external difficulties, illness, a state of prolonged extremeness, hunger, etc. reduce the adaptive potential of the individual, and when faced with a situation that threatens his life goals, maladaptation can occur.

The Holmes and Rage stress tolerance test revealed that 41% of the subjects had low resistance to stress, which indicates that people with sleeping sickness amid a general depletion of the body have little power to deal with stress. 47% have threshold resistance, which means an average degree of stress. This indicates that the stress resistance of the subjects decreases with increasing stressful situations. In this regard, repeated cases of sleeping sickness will gradually reduce resistance to stress. And only 12% of the respondents have a high level of resistance to stress, which indicates the good adaptive capabilities of the body of these people, and that a person has the resources to cope with stress and adapt to new conditions of his life.

Discussion

At the beginning of the twentieth century, despite the brilliant work of M.M. Manaseina, the opinion of sleep as an important process that deserves to be studied no less than wakefulness was still not recognized. However, the events of World War I drew attention to the problem of sleep, and its passive nature was called into question. At the end of this war, the world was struck by an unprecedented epidemic of a mysterious disease. In 1917, several civilians with various neurological impairments were admitted to a psychiatric clinic in Vienna where an outstanding neurologist and neuroanatologist Konstantin von Economo worked. K. von Economo noticed in seven of them a common symptom - uncontrollable drowsiness, and concluded that, despite the differences in symptoms, they all suffer from the same previously unknown disease, which he called encephalitisletargica. This “sleeping sickness” developed as a result of the penetration of a certain virus into the brain, the nature of which remained unknown.

Furthermore, K. von Economo described another 13 such cases. The disease arose as an epidemic,

sometimes in limited groups. She was characterized by high fever, impaired consciousness, visual disturbances, convulsions, and other neurological symptoms. Most of the patients examined by K. von Economo suffered from insurmountable drowsiness, the smaller - from insomnia, inability to sleep. Having carefully studied the pathological material, the scientist came to the conclusion that the “center of wakefulness”, the destruction of which by the virus caused “sleeping sickness”, is located at the level of the junction of the trunk and the diencephalon, and the “center of sleep”, the defeat of which caused insomnia, is in the anterior hypothalamus.

Table 2.

Comparative analysis of the disease

Name	**Esil region**					**Kalachi vi**		
	2010	**2011**	**2012**	**2013**	**2017**	**2010**	**2011**	**2012**
Population	28897	26915	26765	26572	26307	700	637	623
Смертность общая, случай	13,7	15,3	15,2	11,5	5,9	13,4	15,7	13,5
Death rate of onco-pathology	10,7	15,2	17,1	13,5	4,8	28,5	15,6	16
Incidence registered for the first time in life	48313,0	56362,6	54362,0	52601,0	22898,0	36143,0	43956,0	40128,4
Average life expectany in years	61,8	60,7	62,7	64,2	62,7	73,5	73,1	67,5

In the anterior hypothalamus, the GABAergic “slow sleep center” is localized, and in its middle part there are orexinergic neurons responsible for the “correct” inclusion of the “fast sleep center” [1, pp. 581-585].

Japanese scientists working at the University of Texas at the United States found two peptides closely related in structure to the hypothalamus, which they called orexin A and B (from Greek Orexis - appetite) [2, pp. 1-3], [3, pp. 320-322]. It soon became clear that hypocretins 1 and 2 and orexins A and B are the same substances, oligopeptides: orexin A contains 33 amino acid residues, and B - 28. Orexin A has a folded (loop-like) conformation held by disulfide bridges. Orexin B has a linear structure. The orexin A molecule is quite stable, while orexin B rapidly breaks up when administered from the outside [4, pp. 34-41]. Orexinergic cells are very few. By the way, their axons branch strongly, innervating many cells in the cortex and activating brain systems that secrete all the major mediators: acetylcholine, glutamate, GABA, brain amines [5, pp. 1480-1482].

Conclusion

In conclusion, in order to clarify the causes of sleeping sickness, we conducted a literature review of world data on the study of the disease "sleeping sickness". Not only mediators, but also many factors, such as stress, chemical toxins, radiation, viral infections, abiotic factors, etc. affect the functioning of the sleep-wake regulation system. To conduct studies, trip schedules were compiled and expeditionary surveys were conducted along 6 routes. Kalachi and environmentally disadvantaged settlements of the Karaganda and West Kazakhstan regions of the Republic of Kazakhstan. Data obtained using modern methods and technologies showed that the content of heavy metals Pb and Cd ions in the water taken near Kalachi exceeded the MPC level. The content of HM in the soil in samples taken near old mines in the village and on the field did not exceed the MPC level. Studies of biomedical indicators have revealed a violation of carbohydrate metabolism (an increase in the content of total amylase), a slight decrease in the content of hemoglobin, and an increase in the content of low molecular weight organic compounds such as total creatinine and urea, which may be markers of "sleeping sickness" in patients.

The research results did not reveal radiation anomalies in the territory of the settlement and in the premises as well as in the source of drinking water supply. According to the results of a psycho-physiological study, the presence of different levels of stress in the subjects was revealed. Many examiners complained of memory impairment, severe fatigue during physical exertion, distracted

attention and express uncertainty about tomorrow. According to the results of anatomical studies, structural changes in biometric parameters in the aboveground organs of the studied plants were revealed.

Список сокращений

Acat – aspartate aminotrasferase;

Alat – alanine aminotransferase;

ANS - autonomic nervous system;

AP – adapative potential;

CM - congenital malformation;

DCS - diseases of the circulatory system;

HRV - heart rate variability;

ROS – reactive oxygen species;

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