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Use of fluorescent optical fibre probe for recording parameters of brain metabolism in rat model

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ABSTRACT

This study was carried out on groups of clinically healthy male Wistar rats. Animals received distilled drinking water ad libitum for 1 month, water containing succinic acid, water containing zinc sulphate and succinate zinc. Using the method of fluorescence spectroscopy, the parameters of brain metabolism *in vivo* in a model of laboratory rats was investigated. Based on data obtained by fluorescence spectroscopy, we have registered a change in the degree of cellular respiration in different structures of the cerebral cortex with the toxic effect of zinc compounds and succinic acid on the oxygen exchange process.

Keywords: zinc compounds, succinic acid, zinc sulphate, brain metabolism, fluorescence spectroscopy, blood microcirculation, optical measurements in vivo, neurodegenerative diseases

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1. INTRODUCTION

Changes in metabolism in the structures of the brain are noted in many neurodegenerative diseases, generally in old age. Neurodegenerative diseases are a group of slowly progressing pathological states of the nervous system. They are characterised by the death of nerve cells, which leads to the development of specific neurological symptoms. This can occur at different ages but is mainly observed in older people.

It is a well-known fact that disruption of metabolic processes in brain structures are observed long before clinical manifestations. That is why the development of evaluation methods for metabolic disturbances in the brain presents itself as a real problem in fundamental medicine. The free form of zinc (Zn²⁺) in the brain is present in the synaptic vesicles on the terminals of the glutamatergic nerves and is synaptically released during the activity of the neurons. Zn²⁺ is also associated with metalloproteins and is intracellularly mobilised under oxidative stress. Zn²⁺ plays a dynamic role in the physiology and the pathophysiology of brain function.

According to literature^{1,2}, an increase in the concentration of zinc compounds can trigger a pathogenic molecular process leading to the development of neurodegenerative diseases of the central nervous system^{3,4}. Also, exceeding the concentration of zinc in the body can cause disturbances in immune functions and provoke a shortage of chemical compounds, including iron or copper. Excess of zinc compounds for a long period can lead to the emergence of degenerative processes in the pancreas, liver⁵ and organs of the gastrointestinal tract.

At the same time, antioxidant therapy with succinic acid⁴ is capable of providing a neuroprotective effect in the development of neurodegenerative diseases. Succinic acid is an endogenous intracellular metabolite of the Krebs cycle

that performs a catalytic function. This acid increases the rate of reactions and stimulates the formation of ATP. Moreover, there is an antihypoxic effect of succinic acid due to an increase in the concentration of GABA in the brain tissue. Succinic acid helps to regulate cellular metabolism in pathological conditions, accompanied by oxidative stress. Also, the effect on brain tissues of small doses of a zinc succinate compound during its long intake into the organism is of particular interest.

One of the promising directions for studying the parameters of brain metabolism *in vivo* in a model of laboratory rats, is the recording of the spectrum by fluorescence spectroscopy⁶⁻⁸. This method is based on the excitation of the endogenous and exogenous fluorophores of biological tissue and registration of their fluorescence⁹. The method of fluorescence spectroscopy also has a high sensitivity and makes it possible to estimate the intensity of metabolic processes¹⁰, the state of oxygen metabolism of tissues and the distribution of fluorescent nanoparticles within the body¹¹.

This study aims to obtain the results of changes in metabolic processes in the cerebral cortex of rats *in vivo* under conditions of introduction of zinc sulphate, succinic acid and succinate in the drinking water.

2. MATERIAL AND METHODS

Experimental studies were performed on clinically healthy male Wistar rats at the age of 5 months ¹²(n = 6 in the group). Rats were obtained from the FSBI SCBT FMBA of Russia ("Andreevka" branch). Before the transfer of the animals to the clean zone of the vivarium and the beginning of the experiment, the animals were kept in quarantine for 14 days. When placed in quarantine, the veterinarian conducted a primary assessment of the animals' condition. Immediately before the transfer to a clean zone and the formation of experimental groups, the veterinarian conducted a clinical examination of the animals. In the study, animals were selected with no signs of health related abnormalities, so that the average body weight did not differ statistically between the groups. Each animal was assigned an individual number. The basic rules of maintenance and care corresponded to the standards set out in the sanitary rules for the arrangement, equipment and maintenance of experimental biological clinics (Vivarii) and the position-guidance "Laboratory animals". All procedures for routine animal care were carried out by the SOP of the CJSC "Retinoids".

Animals received distilled drinking water ad libitum for 1 month (group 1), water containing succinic acid at a dose of 25 mg per litre (group 2), water containing zinc sulphate at a dose of 3 mg per litre (group 3) and zinc succinate in a dose of 100 mg per litre (group 4). The manifestation and severity of pathological features were evaluated according to the corresponding standard procedure.

For the detection of fluorescence signals, excitation at 365 nm and 450 nm was used, which corresponds to the excitation wavelengths of the NADH and FAD. The fluorescence spectroscopy system with fibre-optical probe "LAKK-M" (SPE "LAZMA" Ltd, Russia) was used for the *in vivo* measurements (fig.1 a). The system provides multiwavelength excitation, detects emission, and processes the fluorescence signal. Its light sources include fluorescence excitation in UV (wavelength = 365 nm, power = 1.5 mW), blue (wavelength = 450 nm, power = 3.5 mW) and green light (wavelength = 532 nm, power = 4.5 mW). The abovementioned fluorescence excitation powers are provided at the tip of fibre probe, which induces an excitation light flux in the tissue of no more than 0.16 W m⁻² for 365 nm and 0.37 W m⁻² for 450 nm. The spectrometer was a polychromator with a diffraction grating, and a CCD (TCD1304AP, Toshiba, Tokyo, Japan) was used as the detector.

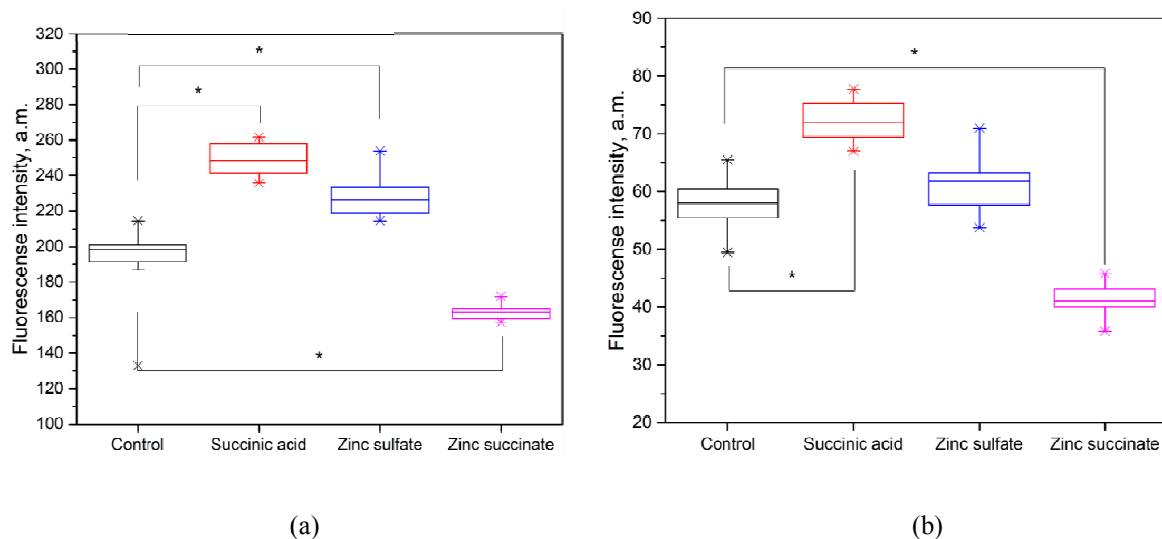


Figure 1. Experimental equipment: scheme of the "LAKK-M" device (a) and the areas of cortex observation (b)

The zones of the motor and visual cortex of rat brain were chosen¹⁵. Around 10 signals per region were recorded from the “LAKK-M” device on the surface of the cerebral cortex for each hemisphere, at an interval of 5-10 seconds. (fig 1).

3. EXPERIMENTAL RESULTS AND DISCUSSION

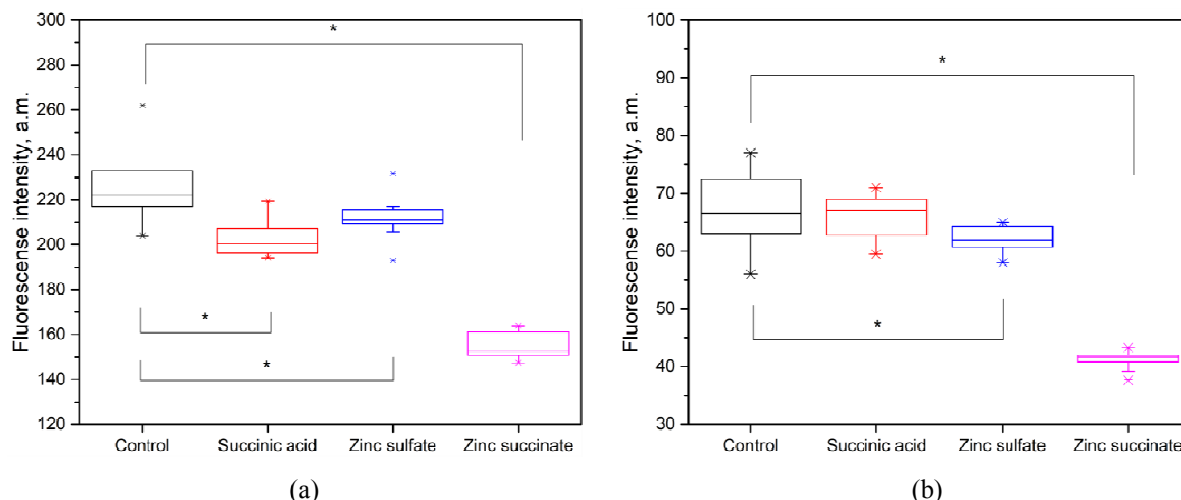
This choice of the excitation wavelengths is based on the fact that in probing biological tissue by UV light (e.g. 365 nm) the endogenous NADH fluorescence excitation is observed in the range 490-510 nm¹³. When excited with blue light (e.g., 430-450 nm) fluorescence flavins are recorded in the range of about 510-520 nm¹⁴. The recorded spectra from each hemisphere were averaged for each group of animals. Then, a comparison was made of the maximum fluorescence intensity parameter at selected wavelengths in the groups.



* - Confirmed the statistical significance of differences in the Mann-Whitney test ($p < 0.05$)

Figure 2– the motor cortex on the wavelength 365 nm (a) and 450 nm (b)

When zinc sulphate and succinic acid were introduced into the drinking water separately at a wavelength of 365 nm, an increase in fluorescence intensity was observed in the region of the motor cortex of the brain, which corresponds to the fluorescence of NADH (fig 2). When zinc succinate is used, the intensity at 365 nm decreases noticeably. At 450 nm, an increase in fluorescence intensity for groups 2 and 3 was observed. For group 4, which received zinc succinate, there is also a noticeable sharp decrease in the fluorescence intensity of FAD.



* - Confirmed the statistical significance of differences in the Mann-Whitney test ($p < 0.05$)

Figure 3 - the visual cortex on the wavelength 365 nm (a) and 450 nm (b)

In the visual cortex of the brain (fig 3) at a wavelength of 365 nm with the consumption of succinic acid, the fluorescence intensity decreased compared to the control group. When measuring at 450 nm, an increase in fluorescence intensity was recorded, which corresponds to a decrease in NADH concentration and FAD growth. Perhaps this is due to the influence of succinic acid on the mitochondrial complex of the II respiration cycle.¹⁶

Presumably, an increase in the intensity of NADH fluorescence¹⁷ corresponds to a worsening of oxygen exchange and cellular respiration in the motor and visual regions of the cerebral cortex for groups 2 and 3. On the contrary, for group 4, there is a sharp decrease in the fluorescence intensity of coenzymes NADH and FAD simultaneously in selected regions^{18,19}. However, when the zinc succinate complex is used, a fluorescence intensity drop at all wavelengths is observed.

Perhaps succinic acid enhances the toxic effect of zinc, and it leads to the suppression of metabolic processes in the brain tissues. By some indications, this reaction is the initial stage of apoptosis of cells. The accumulation of intracellular Zn^{2+} contributes to damage to neurons in certain areas of the brain, including the cortical zones. Excess of zinc is loaded into the mitochondria and leads to its dysfunction. It can be assumed that the main negative effect on neurons due to the accumulation of zinc will be localised in the mitochondria²⁰. The Zn^{2+} ion can cause deep mitochondrial dysfunction and provoke depolarization of mitochondria and formation of reactive oxygen species (ROS)²¹.

4. CONCLUSION

Thus, based on data obtained by fluorescence spectroscopy, we can assume a change in the degree of cellular respiration in different structures of the cerebral cortex with the toxic effect of zinc compounds and succinic acid on the oxygen exchange process. With the use of succinic acid, the change in metabolic processes was presumably associated with an increase in the concentration of coenzymes FAD compared to the control group, which indicates the activation of metabolic processes.

The use of zinc leads to changes in the brain tissues with an increase in coenzymes NADH also a decrease in motor function. Zinc contributes to the death of neurons, because it affects several systems. The excess of zinc can cause deep mitochondrial dysfunction. It can also provoke the depolarisation of mitochondria and the formation of reactive oxygen species (ROS).

Moreover, the introduction of a zinc succinate compound into the rat organism, presumably, could lead to profound metabolic dysfunctions and impaired cellular respiration in brain cells. These changes were recorded by fluorescence spectroscopy in the areas of cerebral cortex of Wistar rats. The results of this work can be used in researches of neuronal death related to major neurological disorders in humans, including stroke, epilepsy and Alzheimer's disease.

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