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Experimental substantiation of the use of highly mineralized bromine chloride sodium water to correct the functional-metabolic continuum in rats with a model of distress

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Abstract

The authors studied the state of the functional-metabolic continuum in white rats while modeling psycho-emotional distress. They evaluated the effect of mineral water with a high content of sodium bromine and chlorides, when applied externally, on the state of the body of the test animals in distress. The model of distress in rats is characterized by rearrangements of metabolic reactions: an increase in endogenous intoxication, an increase in the activity of lipid peroxidation against the background of a decrease in the activity of antioxidant defense

enzymes, a reduction in the content of creatinine and urea, a decrease in the energy supply of the activity of transmembrane metabolism and alanine aminotransferase (ALAT) and aspartate aminotransferase (AST). Simultaneously with adverse changes in metabolic reactions, signs of dystrophic changes in internal organs are determined. Course external application of mineral water (MW) with a high content of sodium, chlorides and bromine in rats with psycho-emotional distress significantly improves metabolism. The normalization of energy metabolism and balance in the lipid-antioxidant system of indicators of endogenous intoxication and the restoration of the functional state of the liver confirms this. In the internal organs, manifestations of dystrophy disappear. The authors believe that using MW improves the condition of regulatory processes, thereby restoring the functional-metabolic continuum in distressed rats.

Keywords: distress; functional-metabolic continuum; bromine chloride sodium mineral water.

Introduction

Due to anthropogenic pressure and the deterioration of social and economic conditions, changes in the human environment cause a constant long-term and robust influence of psychological stress - distress (strong long-term stress) [1, 2]. Distress due to the multifactorial mechanism of occurrence and development has a significant negative impact on the human body since it is a process with a set of rearrangements that deplete the mechanisms of regulation, energy metabolism (including lipid cycles) and mechanisms for stopping the formation of superoxide radicals [3, 4]. The development of distress is associated with increased activity and subsequent depletion of the pituitary-adrenaline system (dysregulation), increased catabolism in tissues, increased oxygen demand due to excessive stimulation of energy reactions (metabolic changes), involution of the mimic-lymphatic system (immune disorders), which can lead to a decrease in the resistance and adaptability of the organism [5, 6]. Dysregulation of the central homeostasis systems during distress leads to impaired microcirculation and, accordingly, to hypoxia at the central and peripheral levels and depletion of the body's adaptive resources, which can manifest itself as multiple organ dysfunction [7, 8, 9].

To correct the consequences of distress, it is necessary to use modulators to restore the balance of homeostasis reactions and not cause side reactions. Mineral waters (MW) of different chemical compositions are considered to be such natural nonspecific modulators [10–24].

Given the above, **the work aimed** to establish the possibility of using highly mineralized bromine chloride sodium water to correct chronic distress`s structural and functional consequences.

Materials and methods of research

The material of the work was the results obtained during the study of 33 white rats of the Wistar line of outbred breeding, with a body weight of 180-200 g. Work with animals was done in an experimental biological clinic (vivarium) State Institution "Ukrainian Research Institute of Medical Rehabilitation Therapy of Ministry of Health of Ukraine ", Odesa. The studies were carried out following the rules established by the Directive of the European Parliament and the Council of Europe (2010/63/EU). According to the tasks of the work, the animals were ranked into three groups: group 1 (control) - 9 intact animals (the data obtained during their study served as a control); group 2 (12 animals) - rats with a reproduced distress model (without the use of a corrective agent) and group 3 (12 animals) - distressed rats treated with a course of external MW procedures. The model of chronic psycho-emotional immobilization stress with signs of endogenous intoxication (distress) was reproducted by periodic immobilization of the test animals for 30 days by placing rats in a device consisting of individual cells that severely limited their mobility [25]. These cells are placed in a large box through which other animals move. Such conditions are reproduced for 3 hours a day. The emotional component of stress was reproduced by adding situational details to the above method, namely, changing the feeding regime, lengthening daylight hours, and changing the number of animals in one cage (overpopulation). The route of MW entry into the body of animals was transdermal (skin-resorptive). For a course of external procedures for MW, rats of the 3rd group were placed in a unique device, where the animals were placed in individual cases, while the tails of the animals were immersed in test tubes with MW for 2/3 of the length (the tail is 5% of the body surface). The MW temperature was maintained within 38-40 ºC. The daily exposure lasted 2 hours; the course consisted of 5 procedures with an interval of 1 day from the 15th to the 30th day of the experiment. The duration of the experiment was 30 days.

The assessment of the formation of endogenous intoxication (EI) was carried out by biochemical methods to verify the content of medium-weight molecules (MWM), namely their fractions - MWM254 (hydrophilic) and MWM280 (lipid-soluble). The level of MWM depends on the body's metabolism and is a prognostic criterion for metabolic disorders. Serum creatinine and urea levels were also determined. To assess the state of the system of lipid

peroxidation and antioxidant protection (LPO/AOD), the content of malondialdehyde (MDA) and catalase activity were determined.

The content of seromucoids, total protein, and its fractions were also determined. The state of the system of energy-dependent transmembrane transport in the liver homogenate was studied by determining the activity of Mg^{2+}-Ca^{2+}-ATPase and Mg^{2+}-Na^{+}/K^{+}-ATPase. To assess the functional activity of the liver, the activity of transamination enzymes, alanine aminotransferase (AlAT) and aspartate aminotransferase (AsAT), was determined in the blood serum. The content of total bilirubin and its fractions was also determined.

The liver is the site of metabolism of chemicals and biological components. Therefore, during the study of the functional state of the liver in some cases, researchers have limited display of the earliest signs of sensory disturbances and its functions. Neurogenic and hepatotropic effect of the studied MW was found by a method of "metabolic tests" scheme Speranskii using barbiturates (thiopental sodium) [26]. Animals sleep time was taken into account after entering the barbiturates, which is an integral test of the MW impact on the functional state of the CNS. Accelerated time of animals falling asleep, compared with previously removed the source of the same background white rat is considered as a manifestation of MW sedative influence on central nervous system, and increased sleep time – as an exciting effect of MW. Duration of sleep medication is associated with the work of the liver, its antitoxic ability, the ability of hepatocytes to reduce the concentration of sodium thiopental in the blood, from which animals wake. General mechanism of monooxygenase system provides biotransformation in the liver and the detoxification of toxins or metabolization of xenobiotics (barbiturates) with subsequent excretion of the liver. The indicators of the negative impact of the investigational product in function of the liver is to increase the effective liquid duration – due to inhibition of inactivation. Increased sleep duration indicates a decrease in detoxifying the liver, and fell – the rase of stimulation of its functional state. The animals were injected with sodium thiopental at a dose of 80 mg/kg.

At the end of the course, the animals were taken out of the experiment by decapitation under ether anesthesia. For biochemical studies, 5 ml of blood was taken from rats. At autopsy, two liver, stomach, heart, and kidney pieces with a volume of 1 cm^3 were removed from the rats. The first piece was fixed for 24 hours in a 4% paraformaldehyde solution, passed through alcohols of increasing concentration, and poured into celloidin. From the obtained blocks, histological sections of 7-9 μm thick were made, stained with hematoxylin-eosin, according to the Van Giesonon technique. The obtained preparations were used for microscopic studies of structural changes in the liver. The second piece was frozen with dry

carbon dioxide (- 70 ° C), histochemical reactions were performed on the prepared cryostat sections to determine the activity of succinate dehydrogenase (LDH) and lactate dehydrogenase (LDH) according to Lojda Z.'s recipe. The enzyme activity was evaluated in conventional units of optical density (c.u.). The methods used are given in the guidelines and approved by the Ministry of Health of Ukraine [27].

Statistical processing of the data obtained in a series of experiments was carried out using biomedical research Statistica and Exel programs, mean values and standard errors were calculated (M ± m). According to the Student's tables significant shifts were considered those within the confidence limits, less than < 0.05.

The obtained data were compared with the corresponding indicators of intact rats (1 control group).

The study used natural water from well No. K1656-g ("Starobelsk Regional Physiotherapeutic Hospital", Starobelsk, Luhansk region, Ukraine), which, according to its physicochemical characteristics, is highly mineralized sodium chloride bromine. The total mineralization of MW is 19.20 g/l, the content of chlorides is 11.82 g/l, the content of bicarbonates is 0.1271 g/l, the content of sulfates is 0.0062 g/l, the content of sodium and potassium is 5.93 g/l, calcium content - 0.9367 g/l, magnesium content - 0.3729 g/l. In the chemical composition of MW, biologically active components and compounds are determined (this is the bromine in high concentrations and much lower concentrations - boron and silicon), which are normalized in balneology and give specific properties to waters [28]. The content of bromine in the form of bromides is 44.80 mg/l (with a balneological norm of more than 25.0 mg/l), the content of boron in the form of orthoboric acid is 19.37 mg/l (with a balneological standart of more than 35.0 mg/l), the silicon content in the form of methylsilicic acid is 12.37 mg/l (with a balneological norm of more than 50.0 mg/l) [28].

Results and discussion

The results of the study of indicators of the state of various aspects of metabolism in rats with distress are shown in Table 1. The development of distress is accompanied by an increase (significant) in the activity of ALT and AST, which may indicate a deterioration in the quality of hepatocyte cell membranes and the release of enzymes into the plasma, as well as an increase in the need for detoxification liver activity Also increases the content of total bilirubin by 56% due to the redistribution of the quantitative composition of its fractions. The range of indirect and direct bilirubin increases by 38% and 21%, indicating a decrease in the activity of conjugation processes and, accordingly, the liver`s detoxification function.

The fact that an unfavorable restructuring of metabolism accompanies the development of distress is evidenced by an increase in the content of urea and creatinine (increased catabolic processes in muscles and nitrogen-containing compounds). In addition, this is evidenced by a significant increase of 73% and 27% in the content of medium-weight molecules - MWM_{254} and MWM_{280}. That is, there is an accumulation of toxic metabolites, which are the basis for the development of EI. An increase in the concentration of MWM, in turn, affects the intensity of the decomposition of biological substrates, which, against the background of a decrease in the excretory function of the hepatorenal complex, reveals the degree of impaired functioning of the body as a whole [29].

Table 1. Biochemical indicators of rats with distress under the influence of external application of MW, M ± m

Blood parameters	1st group	2nd group	P_1	3rd group	P_2
AlAT, O/l	133,09±4,68	148,49 ±4,68	<0,01	91,85 ± 1,72	<0,01
ASAT, O/l	278,84 ± 6,57	470,74 ± 4,77	<0,01	229,23 ± 9,73	<0,01
Index of Ritis, c.u.	2,10 ±0,07	3,22 ±0,12	<0,01	2,50 ±0,84	>0,5
Total bilirubin, μmol/l	5,44 ±0,81	8,50 ±0,97	<0,01	4,83 ± 0,33	>0,5
Direct bilirubin, μmol/l	1,98±0,32	2,40 ± 0,25	>0,5	1,99 ±0,09	>0,5
Indirect bilirubin, μmol/l	3,81±0,51	6,10 ±0,72	<0,01	2,84 ± 0,27	>0,5
Creatinine, μmol/l	47,80 ±0,63	57,43 ± 2,84	<0,01	50,70 ± 1,59	>0,5
Urea, mmol/l	2,80±0,27	3,27 ±0,19	<0,01	4,24 ±0,12	<0,01
Mg^{2+}-Ca^{2+}-ATPase, mg P/g tissue	9,11 ±0,93	4,68 ±0,21	<0,01	8,74 ±0,18	>0,5
Mg^{2+}-Na^{+}/K^{+}-ATPase, mg P/g tissue	6,40 ± 0,62	2,00 ±0,10	<0,01	4,08 ±0,21	<0,05
MWM_{254}, c.u.	0,34 ± 0,02	0,59 ± 0,02	<0,01	0,43 ±0,01	<0,01
MWM_{280}, c.u.	0,22 ±0,01	0,28 ± 0,02	<0,01	0,25 ±0,01	<0,01
MDA, nmol/(min-mg)	5,94 ±0,21	8,54 ±0,32	<0,05	7,76 ± 0,27	<0,05
Catalase, %	76,7 ± 1,52	51,37 ± 1,88	<0,05	72,50 ± 1,55	>0,05

Notes: 1. P_1 – reliability of comparison between 1st and 2nd groups. P_2 - reliability of comparison between 1st and 3rd groups.

At the same time, the activity of ATPases in the liver homogenate is significantly reduced; Mg^{2+}-Ca^{2+}-ATP-ase by 44%, and Mg^{2+}-Na^{+}/K^{+}-ATP-ase by 69%, which indicates a weakening of the active transmembrane transport of substrates and causes the activation of alternative energy-producing reactions. Lipid peroxidation is activated - the content of MDA increases by 30.5%, and the activity of catalase decreases by 33%. The formation of this imbalance can cause damage to cell membranes, including hepatocytes.

An additional assessment of the state of the liver`s detoxification system was carried out using a thiopental test. The results of this study are shown in Table 2. In rats with a model of distress, the time to fall asleep after administering thiopental almost did not change compared to the control. At the same time, the duration of drug-induced sleep nearly doubled, which indicates a weakening of the liver`s detoxification system, possibly due to its depletion.

Table 2. The influence of MV on the detoxification activity of the liver of rats with distress according to the data of the thiopental test, M ± m

Indexes	1st group group	2nd group	P_1	3rd group	P_2
Time to fall asleep, min	2,88 ±0,26	2,67 ± 0,05	> 0,5	3,15 ±0,87	> 0,5
Duration of sleep, min	53,17 ± 0,66	87,01 ±0,24	<0,01	50,90 ± 0,31	> 0,5

Notes: 1. P_1 – reliability of comparison between 1st and 2nd groups. 2. P_2 - reliability of comparison between 1st and 3rd groups

To obtain a complete picture of changes in the test rats` organs, we studied of the morphological picture of the stomach, heart, liver, and kidneys. The results of these studies showed the following.

In the stomach, there was a breastiness of the cytoplasm of the epithelial cells of the glands, the presence of vacuoles in some of them, and edematous distribution of the interstitial layers of the mucosa. The goblet cells are reduced in size. SDH activity in epitheliocytes decreased compared with the control, in some areas, it was (6.0 ± 0.1) c. u., and in some - (5.0 ± 0.12) c. u. LDH activity was relatively high (6.0 ± 0.1) c. u.

In the liver, the lobular organization of the parenchyma was preserved, and the vessels of the triads and the central vein were of standard blood supply in some lobules - plasma stasis. Hepatocytes are collected in short beams in the center of the lobule; in the rest, they are arranged randomly. Medium-sized hepatocytes with dark eosinophilic cytoplasm. Many hepatocytes with small vacuoles in the cytoplasm. The activity of SDH in them is (5.0±0.1) c. u., LDH activity - (5.0 ± 0.20) c. u. That is, the activity of the enzymes SDH and LDH is reduced, which indicates signs of inhibition of the activity of redox processes in the liver.

In the heart, the layered and bundled organization of the myocardium remains unchanged. In cardiomyocytes, fuzzy transverse shading and oval enlarged nuclei are observed. The activity of SDH in them is (7.0±0.1) c. u., LDH activity - (7.0 ± 6.0) c. u. in different parts of the myocardium.

In the kidneys, attention was drawn to an increase in Bowman's spaces and swelling of the epithelium of the tubules up to the closure of its lumen and vacuoles in some of them. The activity of SDH in the epithelium of the tubules was (7.0±0.1) c. u., and LDH activity - (7.0 ± 0.12) c. u.

In the internal organs there are signs of debilitating functional activity and the development of dystrophic processes.

Studies conducted after completing the course of correction of MW procedures identified positive changes in metabolic parameters and morphological characteristics of internal organs.

According to Table 1, the activity of ALT and AST significantly decreased in comparison with uncorrected distress and the control. It can be assumed that the prevalence of damage to hepatocyte membranes decreases, so the output of these enzymes also decreases. The Ritis index is approaching the control data. The creatinine content is restored, but the range of urea retains a tendency to increase; that is, the intensity of the catabolism of nitrogen-containing compounds remains enhanced, despite using the course of MW.

The activity of Mg^{2+}-Ca^{2+}-ATPase and Mg^{2+}-Na^{+}/K^{+}-ATPase increased almost twice that of the rats with uncorrected distress but did not reach the control level. In addition, there is an imbalance in their activity. This fact can be interpreted as the persistence of the lack of energy supply for transmembrane transport, which requires the increased activity of other energy generation pathways. This is evidenced by preserving of a sufficiently high LPO activity, although it decreased compared to the 2nd group of rats (with uncorrected distress). Attention should be paid to the increase in catalase activity, which indicates an improvement in the protection of cell membranes and, accordingly, an improvement in the activity of the functional systems of the body of the test rats.

Normalization of metabolic processes leads to a decrease in endogenous intoxication. This applies to a greater extent to the MWM280 fraction (lipid-soluble substances), to a lesser extent, to MWM_{254} (hydrophilic). There is a decrease in the content of total bilirubin to the normal range, especially its indirect fraction, which indicates signs of restoration of the activity of the liver`s detoxifying function. This correlates with the data on the thiopental test (Table 2).

The sleep duration of the animals that received the MW course is restored; it decreases to the control level, indicating the normalization of the liver detoxification function.

Simultaneously improving the course of metabolic processes in rats of the 3rd group under the influence of MW, the morphological picture of the internal organs also enchances.

In the stomach of the test rats, the histological picture corresponds to that of control animals; that is, vacuolization of epithelial cells of the glands of the gastric mucosa disappears, swelling of the interstitial layers, and the activity of SDH and LDH increases. In the liver, there are no phenomena of plasma stasis, disordered arrangement of hepatocytes, and their vacuolization; this also increases the activity of SDH and LDH.

The histological picture of the myocardium and the activity of redox enzymes in it is typical. The morphological characteristics of the cortical and brain tissue of the kidneys are also normalized. In general, under the influence of the external application course of MW, the manifestations of dystrophic changes in the studied organs, which are formed during the development of the distress model in rats, decrease and even disappear.

Thus, the results of the studies determined that changes in metabolism accompany the development of psycho-emotional distress in the test rats. First, the activity of the main energy-forming processes and alternative reactions of energy production changes; secondly, detoxification processes are activated, and their imbalance is formed. Obviously, in this case, metabolites accumulation occures since the indicators of endogenous intoxication increase. Metabolic rearrangements are closely related to or cause the development of dystrophic changes in the structure of internal organs; that is, we observe manifestations of the functional metabolic continuum [30].

Using a course of external procedures with MW in rats with psychoemotional distress improves some reactions to the normalization of metabolic processes. At the same time, structural manifestations of dystrophy disappear in the parenchyma of internal organs, and normalization of the activity of redox enzymes is determined histochemically. Psycho-emotional stress is a consequence of continuous or periodic long-term exposure of the body to negative emotional factors. Adaptation of the body to the effects of these factors occurs due to changes in physiological constants while maintaining the level of metabolic processes [30, 31]. Numerous chemical processes necessary to adapt the body to new conditions of existence lead to the formation of a certain amount of undissolved toxins, which provokes a loss of tissue elasticity (including the vascular wall), which leads to changes in hemodynamics and promotes restructuring in the parenchyma of internal organs. The degree of resistance of the

body to a stress factor is determined by the functional state of the hypothalamic-pituitary-adrenal system [32, 33].

The use of bromine highly mineralized sodium chloride water under these conditions, containing bromine in a sufficiently high concentration (the main biological effect of which is to restore the balance of activating and inhibitory processes in the central nervous system), clearly reduces the destructive impact of the imbalance of these processes in animals. In addition, the use of MW has a positive effect on metabolic processes, which prevents the development of dystrophic changes in the parenchyma of internal organs and contributes to the restoration of the functional metabolic continuum.

That is highly mineralized sodium chloride water, which contains bromine as a specific biological component, plays the role of a non-specific modulator.

Conclusions

Thus, the obtained results allow us to consider that using highly mineralized bromine chloride sodium water in the form of external procedures significantly improves the state of regulatory processes and contributes to restoring the metabolic structural continuum in rats with a model of distress.

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