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EVALUATION OF THE INFLUENCE OF CHLORIDE SODIUM RAPE ON THE EXTERNAL APPLICATION ON THE STRUCTURAL AND FUNCTIONAL CONDITION OF RATS KIDNEY ON DEVELOPMENT

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Abstract

Much attention has been paid to the study of the numerous effects of chronic stress and the methods of their correction, but some questions remain unanswered. Goal. To investigate the effect of highly mineralized sodium chloride mineral water (brine) on the structural and functional state of rat kidneys with experimental distress. White female rats weighing 180 - 220 g were ranked into three groups: group I intact rats (control), group II rats with a distress model (duration 30 days); group III - rats with a model of distress, who from 15 to 30 day of experiment received a course of external procedures with rapes. In group II rats, the development of distress caused dystrophic changes in the kidneys, which led to inhibition of their urinary, ion-regulating and excretory functions. To correct the manifestations of distress rats received a course of external procedures with brine (5 procedures for 2 hours a day). Renal function restored: GFR exceeds group I data by 30%, resulting in complete recovery of the diuresis. Urea excretion is restored, and creatinine excretion exceeds the control level by 30% ($p < 0.001$). The concentration of potassium ions is restored to the control level, their excretion is reduced by 20%, and the urinary excretion of sodium and chloride ions is increased by — 90% and 57%. Microscopically ─ the structure of nephrons and their components without visual changes, that is, does not differ from the first group of rats. Conclusions. Studies have shown that with transdermal administration of sodium chloride brine has a significant protective effect on the course of pathological changes in the function of the kidney of rats against the background of the development of distress.

Key words: distress, functional state of kidneys, brine.

Introduction. Sodium chloride mineral waters (SCMW) are the most famous waters used in balneology [1, 2]. They are widespread and are characterized by certain therapeutic properties, which are manifested in total mineralization from 8 g/l [3, 4]. It should be noted - the presence of biologically active components and compounds, such as bromine, silicon, boron, iodine, etc., as part of the SCMW. give water specific properties [5, 6]. Cardiovascular, central and peripheral nervous system diseases, diabetes of the second type, etc., in the pathogenesis of which one of the leading components is given to chronic stress are indications for the use in the clinical practice of SCMW [1 — 4]. Chronic stress affects virtually all functions and levels of organization of the body, from systemic to molecular [8, 9, 10]. It has been experimentally confirmed that during chronical stress, the mass of the spleen and thymus decreases, and changes in the immune system are observed. Catecholamines (due to activation of the sympathetic nervous system and brain part of the adrenal glands), as well as endorphins and enkephalins [9].

Renal dysfunction has been determined (urinary and excretion processes are suppressed), which, in combination with the depletion of adaptive systems of the body, impaired balance in the lipid peroxidation - antioxidant system, leads to the accumulation of toxic products of metabolism and the development of the state of endogenous intoxication [11, 12]. It should be noted that despite the widespread use in the balneology and medical rehabilitation of methods of external treatment (baths, compresses, electrophoresis, etc.) and obtaining therapeutic effects, the sanogenetic mechanisms of their impact have not been sufficiently studied [13, 14].

Leather is an excellent protective barrier. The main functions of the skin are the protection of the body from water loss, thermoregulation and protection from the entry into the body of various exogenous substances, including during balneological procedures [15]. It is impossible to fully argue which structural elements of the skin create a barrier to the penetration of exogenous substances. Most likely, this function is performed by all components of the skin. For macromolecules, the boundaries of horn cells are the universal barrier, and for micromolecules they are not a barrier. Importantly, an average of 15 sebaceous glands, 10 hair follicles, 100 sweat glands, 3 blood vessels, 12 nerve endings, and 360 sm nerve fibers account for 1 sm^2 [16]. In addition, the surface area of the skin is about 2 m^2, and the volume of the regional vascular bed is about 1/3 of the total circulation. These circumstances make it possible to consider that the skin is a weighty accumulator and conductor of signals (due to the area and layer of the dermis and the underlying layer of adipose tissue), and the effector organ [17]. Numerous receptors, being in the skin, transform the energy of various stimuli into energy of the nervous (receptor) potential.

The skin is the site of synthesis of a number of biologically active substances (serotonin, histamine, heparin, cytokines, hormones, etc.) [17].

There are common mechanisms of hydrotherapy, which consists of effects on the skin of temperature, mechanical and chemical stimuli. The temperature factor influences blood circulation, nervous system and metabolic processes, redistributes blood. Mechanical bath factor - pressure and movement of water, which enhance the effect of the temperature factor, affect the blood and lymphatic circulation and respiration. The special factor of the baths is the chemical factor. It affects the skin, irritates its peripheral receptors. Once in the body, chemicals affect different systems of the body and metabolic processes, promote the development of protective and adaptive reactions, etc. [1 - 4, 18].

In view of the above, the aim of the work is to investigate the effect of highly mineralized sodium chloride mineral water on the structural and functional state of rat kidneys with experimental distress.

Materials and methods of research. The experiment was performed on 40 white female Wistar rats of an outbred breeding with a body weight of 180 - 200 g. The work was carried out in accordance with the rules established by the Directive of the European Parliament and of the Council of Europe (2010/63 / EU) and guidelines [19]. Animals were classified into three groups: group I control (10 animals) intact rats, group II (15 animals) - rats with distress model; Group III (15 animals) were rats with a distress model who received a course of external CF procedures from 15 to 30 experiments. The pathway of brine to the animal body is transdermal (skin-resorptive). The rats were housed in a special device, with the tails of the animals immersed in CF tubes (the tail was 5% of the body surface). The brine temperature was maintained within 38 – 40 ºC. Daily exposure lasted 2 hours, the course consisted of 5 procedures with an interval of 1 day. The study used a model of chronic psycho-emotional immobilization stress, complicated by situational factors (distress), which was developed in the State University "Ukr. MR Research Institute and Ministry of Health of Ukraine" and protected by Patent [20]. The distress model was reproduced for 30 days. The animal immobilization method (15 × 4 × 5 sm pencil box), which is the only stressor in the simulation of immobilization stress, was supplemented with the placement of pencils in a large cage with many rats moving freely. After the immobilization period (3 hours), the animals were further exposed to situational stressors. One of the following stressors was attached daily without a certain sequence: change of diet and drink, night electric lighting, overcrowding of cells (12 pieces instead of 6), change of composition of animals in cages. Verification of manifestations of distress in rats was carried out on the 30th day from the beginning of the simulation.

The functional state of the kidneys was evaluated by the influence on the function of the urinary tract (glomerular filtration rate, tubular reabsorption, diuresis), on the excretory function (by the excretion of creatinine, urea and chlorides). Ion-regulating function (by concentration and daily excretion of sodium, potassium and chloride ions) was investigated.

Determined the acid-alkaline reaction of daily urine in terms of the concentration of hydrogen ions. According to the results of microscopic studies, changes in the structural organization of the kidneys were determined. Animals from the experiment were removed under ether anesthesia.

The methods used are given in the guidelines and approved by the Ministry of Health of Ukraine, experimental studies were carried out in accordance with legal documents [21]. For all means of processing statistical material, those within the range of the Student's probability were considered as less than <0.05.

In the study, the brine lake of Oleshkiv district of Kherson region was used, which by its physicochemical properties is characterized as highly mineralized sodium chloride, slightly alkaline and corresponds to the following formula:

$$M_{9,98–22,43}\frac{Cl89-94\,HCO_3\,5-8\,SO_4\,1}{(Na+K)90-96\,Mg\,2-5\,Ca\,1-2}\quad pH\,8{,}2-8{,}6$$

During the research period (2016 – 2017), the mineralization of brine varied within 18,14 – 22,43 g/l, the content of chloride ions ranged from 9762,5 mg /l to 12349,0 mg /l, and the hydrocarbonate ions 1170,4 – 1415,2 mg/l, sulfate ions 104,1 – 126,7 mg/l, sodium and potassium ions 6594,4 mg /l – 8282,1 mg/l, magnesium ions 91,2 mg /l – 121, 6 mg /l, calcium ions 110,0 – 130,0 mg /l.

Values of mineralization of brine are not constant values in time and space and change during periods of intense evaporation, feeding of the reservoir due to precipitation, falling of underground sources, groundwater, seawater.

Biologically active components and compounds that are normalized in balneology and give water-specific properties such as bromine, methasilicic acid, orthoboric acid, iodine are found in concentrations below balneological norms (Table 1) [22].

Table 1. Bioactive components and compounds of lake brine. Salt pans

Components and compounds	Content, mg/l	The mass concentration of the component, mg/l, according to GOST 42.10-02-96 not less than type of water
Orthoboric acid (H_3BO_3)	15,15 – 17,05	35,0 borne
Methasilicic acid (H_2SiO_3)	9,14 – 11,18	50,0 silicon
Iodine	0,24 – 0,25	5,0 iodine
Bromine	14,40 – 16,00	25,0 bromine

Results and discussion

On the 30th day of the development of distress, a significant decrease in the functional capacity of the kidneys was established (Table 2). In the analysis of the data, a decrease in the urinary function of the kidneys was found: the volume of diuretic diuretics decreased by 30% due to a 42% decrease in glomerular filtration rate (GFR). Even a significant reduction in tubular reabsorption ($p < 0.001$) cannot increase the volume of diuresis per day. There is a suppression of excretion of nitrogenous metabolic products - the excretion of creatinine and urea is reduced by 42 and 25%, respectively. The daily urine concentration of potassium ions was increased by 40% and the urinary chloride ion concentration and excretion decreased by an average of 20%. Thus, it can be

argued that the development of experimental distress is accompanied by a significant inhibition of the urinary and excretory functions of the kidneys.

In the rats of the third group, which received the course of procedures with the laceration of the lake. Saline, an increase in the rate of filtration of the fluid in the glomerulus of the kidneys by 30%, which, in combination with an increase in the percentage of fluid re-absorption in the tubules, leads to the restoration of the daily diuresis to the level of control indicators.

The daily excretion of urea, which had a significant decrease in the background of the development of distress, under the influence of the course of procedures with rapes increases to the level of control values, and the excretion of creatinine exceeds the level of control by 30% ($p < 0.001$). The concentration of potassium ions is restored to the control level and their excretion is reduced by 20%, the concentration of sodium ions and chloride ions is increased by 144 and 42%, respectively, and their daily excretion by urine - by 90% ($p < 0.001$) and 57%
($p < 0.001$), respectively.

Therefore, the established changes under the influence of sodium chloride brine, indicate its significant corrective effect on the functional status of the kidney of rats with distress.

Table 1 - Functional state of the kidneys of distressed rats and distressed rats under the influence of transdermal intake of brine

Indicators	I group (control)	II group (experimental distress)		D	P	III group (experimental distress and brine)		D	P
	($M_1 \pm m_1$)	($M_2 \pm m_2$)	%			($M_3 \pm m_3$)	%		
Daily diuresis, ml/dm^2 of body surface	1,17 ± 0,10	0,82 ± 0,008	70	– 0,35	< 0,01	1,03 ± 0,08	88	– 0,14	> 0,5
Glomerular filtration rate, ml/(dm^2 × min)	0,12 ± 0,01	0,07 ± 0,001	58	– 0,05	< 0,001	0,16 ± 0,001	130	+ 0,04	< 0,001
Tubular reabsorption, percentage to filter,%	99,27 ± 0,06	98,41 ± 0,06	99,13	– 0,86	<0,001	99,57 ± 0,06	100,3	+ 0,30	< 0,001
The excretion of creatinine, mmol	0,012± 0,001	0,007 ± 0,001	58	– 0,005	< 0,001	0,016± 0,001	130	+ 0,004	< 0,001
Urea excretion, mmol	0,69 ± 0,06	0,52 ± 0,002	75	– 0,17	< 0,02	0,63 ± 0,009	91	– 0,06	> 0,2
pH of daily urine, units pH	6,61 ± 0,12	6,28 ± 0,011	95	– 0,33	< 0,02	6,15 ± 0,011	93	– 0,46	< 0,01
Concentration of potassium ions in daily urine, mmol/l	100,57 ± 2,48	140,80 ± 1,15	140	+ 40,23	<0,001	91,52 ± 4,88	91	– 9,05	> 0,1
Daily excretion of potassium ions, mmol	0,11 ± 0,003	0,10 ± 0,01	95	– 0,01	> 0,5	0,09 ± 0,001	82	– 0,02	< 0,001
Concentration of sodium ions in daily urine, mmol/l	115,58 ± 4,07	127,14 ± 5,74	110	+11,56	> 0,1	282,01± 6,00	244	+166,4	< 0,001
Daily excretion of sodium ions, mmol	0,13 ± 0,003	0,13 ± 0,001	100	0,13 ± 0,001		0,25 ± 0,001	190	+ 0,12	< 0,001
Concentration of chloride ions in daily urine, mmol/ l	262,72 ± 7,79	223,31 ± 2,37	85	–39,41	< 0,001	373,06± 2,37	142	+110,3	< 0,001
Daily excretion of chloride ions, mmol	0,30± 0,01	0,23± 0,002	77	– 0,07	< 0,001	0,47 ± 0,002	157	+ 0,17	< 0,001

Notes: 1. ($M_1 \pm m_1$) and ($M_2 \pm m_2$) are arithmetic averages with error values; 2. D1 is the difference between M_1 and M_2; D_2 is the difference between M_1 and M_3; P_1 is the probability of comparison between M_1 and M_2; P_2 - likelihood of comparison between M_1 and M_3; 100% accepted data from the control group of animals.

Morphological studies on the 30th day of the development of the model of distress revealed that the renal bodies are sparse. The capillary glomeruli of the renal little bodies in their most rounded form, are found glomeruli of rounded shape. Bowman's space is dense, its outer membrane dense, thickened. Intra-renal vessels are stagnant-full-blooded. In the tortuous tubules, the epithelium is entire, the cytoplasm of the epitheliocytes is swollen, eosinophilic, coarse-grained. The nuclei are mostly large, fuzzy, light-colored; in part of the nucleus cells are pictonic. The interstitial layers are thin, but they have single old hemorrhages.

That is, the development of distress causes dystrophic changes in the kidneys, which contributes to the development of endogenous intoxication, a marker of which is a decrease in the excretion of creatinine and urea with urine and increase their content in the blood.

Morphological studies revealed that rats received a microscopic course of external procedures with sodium chloride chloride on the background of the development of distress in the mode of external use, ie the structure of nephrons and their components without visual changes, that is, does not differ from the control group of intact animals.

CONCLUSIONS

1. Against the background of the development of distress in rats, suppression of urinary, ion-regulating and excretory functions of the kidneys was established: the volume of diuretic diuretic decreased by 30% ($p < 0.001$) due to a 42% decrease ($p < 0.001$) of GFR; excretion of creatinine, urea and chloride ions decreased by 42%, 25% and 15%, respectively ($p < 0.001$). The daily urine concentration of potassium ions was increased by 40% and the urinary chloride ion concentration and excretion decreased by an average of 20%. Microscopic studies have determined that the capillary glomeruli of the renal little bodies are rather sparse, some of them lobular in shape, the Bowman space is slender, its outer membrane is

dense and thick. Intra-renal vessels are stagnant-full-blooded. In the tortuous tubules, the cytoplasm of the epitheliocytes is swollen, eosinophilic, coarse-grained. The nuclei are mostly large, fuzzy, light-colored; in part of the nucleus cells are pictonic. The interstitial layers are thin, but they have single old hemorrhages. That is, the development of distress is accompanied by dystrophic changes in the kidneys.

2. The use of external procedures with rapes leads to the restoration of renal function: GFR exceeds the data of the control group by 30%, which leads to a complete restoration of the value of diuresis. Urea excretion is restored, and creatinine excretion exceeds the control level by 30% ($p < 0.001$). The concentration of potassium ions is restored to the control level, their excretion is reduced by 20%, and the urinary excretion of sodium and chloride ions is increased by - 90% and 57%. Microscopically - the structure of nephrons and their components without visual changes, that is, does not differ from the control group of intact animals.

It should be noted that the established effects of the influence of sodium chloride brine during its transdermal application on the course of pathological changes in the function of the kidney of rats against the background of the development of distress testifies to its significant correcting ability. The data obtained substantiate the feasibility of the use of sodium chloride brine in conducting appropriate clinical trials.

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