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FEATURES OF THE REACTION OF THE KIDNEY FUNCTION OF HEALTHY RATS TO THE INTAKE OF BORIC HYDROCARBONATE SODIUM MINERAL WATERS OF VARYING QUANTITATIVE MACRO- AND MICRO-COMPOSITION

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Abstract

The purpose of the work is a comparative assessment of the effect of boron hydrocarbonate sodium mineral waters with a varying quantitative and qualitative composition of macroelements and with different amounts of a bioactive element on the state of the urinary system of rats. **Materials and methods.** The work was performed on 81 white rats of the Wistar line of auto bred breeding, weighing 180-200 g. The rats were divided into four groups: group 1 - 21 intact rats served as controls; group 2 - 20 rats treated intragastrically with a soft probe MW "Polyana Ploskivskaya" at a dose of 1% of body weight daily, a course of 7 days; group 3 - 20 rats treated with MW "Polyana Kupel" according to the above method; group 4 - 20 rats treated with MW "Svalyava" according to the above process. Before removing the animals from the experiment, daily urine was collected from them; blood was also taken from the tail vein for biochemical studies. At autopsy, pieces of kidneys were taken for histological and histoenzymological studies (the activity of succinate dehydrogenase and lactate dehydrogenase was determined). **Results.** The histological studies showed no pathological changes in the kidneys were observed with the use of the studied boron MW. Still, there were structural changes, as well as an increase in the activity of redox enzymes. Changes in kidney function were unidirectional with using any of the investigated MW. Still, they had quantitative differences, which are less related to the amount of boron (in the form of orthoboric acid) in 1 liter of MW and more dependent on the osmolarity of the MW, the qualitative composition of macronutrients, and their ratio. **Conclusion.** We believe that osmolarity, as an integrated indicator of the state of the macronutrient component of MW, can be used to assess its effect on the structural and functional characteristics of the kidneys.

Keywords: boron-containing mineral waters; renal parenchyma; diuresis; excretory function; osmolarity.

INTRODUCTION

Mineral waters (MW) are one of the natural healing factors used to prevent and treat many nosologies [1, 2, 3, 4]. According to modern concepts, MW are nonspecific modulators [5, 6, 7, 8], that is, factors that maintain or restore the coordination and balance of the body's functional systems. From physicochemical characteristics, MW is a system that includes macro- and microelement components [9, 10]. Trace elements are metallic and non-metallic elemental components contained in the corresponding MW in amounts not exceeding a few mg per 1 liter of MW [11, 12]. Trace elements are part of stable complexes with body proteins, and due to this, they realize their bioactivity. Usually, their presence is associated with the biological activity of MW. That is, the role of carriers for the further participation of microelements in the activity of the body is played by blood and tissue proteins that regulate the activity of cells of the nervous, endocrine, and immune systems, which affects the humoral mechanisms that control the entire vital activity of the body [13, 14]. Most trace elements are part of enzymes (active centers), prohormones, and active hormones [15]. The action of one of the trace elements, boron (B), is associated with the effect on the calcium content in the bones, the activity of the cardiovascular and central nervous systems, and the activity of carbohydrate and water-electrolyte metabolism [16–20]. At the same time, little attention is paid to the influence of the macro elemental component of MW, i.e., metallic and non-metallic elements contained in MW in the amount of g/l, on the formation and development of reactions of the functional systems of the body in the available literature. As a rule, only the total number of macroelements and their nomenclature is indicated [21, 22]. At the same time, the quantitative ratio of macronutrients and the features of the action of MW are practically not studied. This approach reduces the quality of the assessment of MW bioactivity.

Accordingly, the purpose of our study was a comparative assessment of the characteristics of the reactions of the excretory system of rats to the use of MW, which differ in the amount of the same macroelements (primarily hydrocarbons and sodium), and the amount of boron as the main microelement.

MATERIALS AND METHODS

The studies were performed on 56 white female rats, Wistar outbred breeding line, aged 6-8 months, weighing 180 - 210. Experimental studies were conducted in accordance with the rules established by the Directive of the European Parliament and the Council (2010/63/EU), by the order of the Ministry of Education and Science, Youth and Sports of Ukraine No. 249 of March 1, 2012 "On Approval of the Procedure for conducting scientific experiments, experiments on animals by scientific institutions" [23, 24]. During the experiment, the animals were in the experimental biological clinic (vivarium) of the State Institution "Ukrainian Research Institute of Medical Rehabilitation and Resort Therapy of the Ministry of Health of Ukraine", Odesa in the conditions of free access to food and water. The animals were kept in standard laboratory conditions: photoperiod - light /darkness 12:12; air temperature - 22 ± 2 ° C; humidity - 55 ± 10% [25].

All animals were randomly divided into four groups:

group 1 - 21 rats that were kept in vivarium conditions and were not exposed to any influences; their data served as control;

group 2 - 20 rats receiving an internal course of dosed administration of MW "Polyana Ploskivska";

group 3 - 20 rats that received an internal course of dosed intake of MW "Polyana Kupel";

Group 4 - 20 rats who received a course of internal administration of MW "Svalyava".

The introduction of MW was carried out every day, for seven days, intragastrically, with a soft probe with olive, at a dose of 1% of the body weight of the rat.

The day before the end of the experiment, daily urine was collected from the animals, for which the animals were placed in special individual boxes (exchange cells). The state of functional activity of the kidneys was assessed by the volume of daily diuresis, glomerular filtration rate (GFR), percentage of tubular reabsorption, excretion of urea, and creatinine with daily urine; urine pH; concentration, and daily excretion of Na^+, K^+, Cl^-. For biochemical studies, blood was obtained from the tail vein. In blood plasma were determined pH, the concentration of Na^+, K^+, Cl^-, urea, and creatinine. The concentration of electrolytes in the blood and urine was carried out using the device AEK-01 "Quer".

Rats were withdrawn from the experiment by decapitation under light ether anesthesia 16–18 hours after the last administration of MW. For histological and histoenzymatic studies, two pieces of kidneys with a volume of 0.5 cm^3 were removed. Histological analyses were performed on celloidin sections. The condition of the renal corpuscles, convoluted and straight tubules, and interstitial layers was studied. To determine the energy supply for the functioning of the nephron components, we evaluated changes in the activity of succinate dehydrogenase (SDH) and lactate dehydrogenase (LDH) on cryostat sections of native kidney tissue according to the prescription of the Lojda manual [26]. Enzyme activity was evaluated in conventional units of optical density (arbitrary units of optical density).

Methodical methods and techniques used in the research were approved by order of the Ministry of Health of Ukraine № 692 of 28.09.2009 [27].

All data were processed using the statistical package Statistica 10.0 (Statsoft/Dell, Tulsa, OK, USA). The descriptive statistics of the data in tables include mean ± standard error of the mean (SEM) or mean ± standard deviation. Significance was assessed by using the one-way ANOVA followed by t-test. Values were considered statistically significant when P value is less than 0.05.

The chemical composition of mineral waters used in the study was as follows:

1. Boron highly mineralized hydrocarbonate sodium water "Polyana Ploskivska" - total salt content – 10.69 g/l. Content of individual elements: (Na^+ + K^+) – 2.92 g/l; Ca^{2+} – 0.096 g/l, Mg^{2+} – 0.015 g/l; CI^- – 0.639 g/l; SO_4^{2-} – 0.0021 g/l, HCO_3 – 7.015 g/l. The boron content in the form of H_3BO_3 was 0.210 g/l, with the balneological norm for boron waters from 0.035 g/l [28]. The content of H_2SiO_3 was 0.020 g/l (at the balneological norm for silicon waters from 0.050 g/l) [28].

2. Boric medium-mineralized sodium bicarbonate MW "Polyana Kupel" - the total salt content was – 8.04 g/l. The content of individual elements: (Na^+ + K^+) – 2.125 g/l, Ca^{2+} – 0.08 g/l, Mg^{2+} - 0.002 g/l; CI^-– 0.502 g/l; H_3BO_3 – 5,322 g/l; SO_4^{2-}– 0.0021 g/l. The H_3BO_3 content was 0.160 g/l. The H_2SiO_3 content was 0.021 g/l.

3. Boric medium-mineralized sodium bicarbonate water "Svalyava" - total salt content – 5.94 g/l. Content of individual elements: (Na^+ + K^+) – 1.44 g/l, Ca^{2+} – 0.168 g/l, Mg^{2+} – 0.0122 g/l; CI^-– 0.092 g/l; SO_4^{2-} – 0.0165 g/l; H_3BO_3 – 4.21 g/l; H_2SiO_3 – 0.0264 g/l. The H_3BO_3 content was 0.100 g/l.

According to the given data, the total salt content in the used MW differed by 1.8 and 1.3 times. The content of the main macroelements also varied, but to a lesser extent; in addition, Mg^{2+} was not determined in the second water. The biologically active element in all studied MW was H_3BO_3, but its amount also differed: in MW "Polyana Ploskivska", it is twice as much as in MW "Svalyava". Certain quantitative discrepancies in the content of the components of the chemical composition can affect the characteristics of the reactions of the excretory system during the course application of MW.

RESULTS AND DISCUSSION

The conducted studies have shown that the course use of the investigated MW impacted the kidneys′ urinary, excretory, and ion-exchange functions of the kidneys. It should be emphasized that, despite the quantitative differences in the content of macro-and microelements, the reaction was unidirectional when using all the studied MWs. According to table 1, the use of each of the MW led to an increase in daily diuresis. However, the quantitative characteristics of this increase differed for the investigated MW: for "Polyana Ploskivska", it was 12,5 %; for "Polyana Kupel" – 19 %; for "Svalyava" – 32 %. It should be noted that the content of H_3BO_3 in the "Svalyava" was the smallest of the three MW.

Table 1. Comparative evaluation of the functional activity of the kidneys of rats using MW with high H_3BO_3 content, M± m

Indicators	Groups			
	1st Group	**2nd Group**	**3rd Group**	**4th Group**
Daily diuresis, ml/dm^3	1,44 ± 0,06	1,62 ± 0,09*	1,72 ± 0,04*	1,90 ± 0,05*
Glomerular filtration, ml/(dm^2×min)	0,11 ± 0,01	0,14 ± 0,003*	0,16 ± 0,003*	0,15 ± 0,005*
Tubular reabsorption, %	98,95 ± 0,09	99,20 ± 0,03*	99,22 ± 0,05*	99,09 ± 0,11
Elimination of creatinine, mmol	0,011± 0,001	0,013 ±0,0005	0,016 ± 0,0001*	0,015 ± 0,0005*
Urea excretion, mmol	0,86 ± 0,05	1,21 ± 0,05*	1,23 ± 0,01*	1,14 ± 0,05*
Urine pH, c.u.	6,62 ± 0,20	6,12 ± 0,08	7,34 ± 0,02*	6,72 ± 0,16

Note: M±m are arithmetic averages with errors of indicators; * - significant changes in indicators of 2nd, 3rd and 4th groups compared with the 1st group ($p<0.05$).

Since the action of boron is associated with the inactivation of energy formation processes, it can be assumed that MW, in which its content is lower, inhibits the activity of urinary formation processes less. Changes in daily diuresis depend on GFR. The use of the studied MW influenced this indicator, increasing it, but quantitatively these changes differed. When using MW "Polyana Ploskivska", GFR growth was 12 %; when using MW "Polyana Kupel" – 45 %, and in the case of "Svalyava" – 36 %.

The second factor in changes in daily diuresis is the percentage of tubular reabsorption (energy-dependent process). The general tendency to increase this indicator was observed for each MW, especially for "Polyana Ploskivska" and "Polyana Kupel", and amounted to 0,25 % for each; for "Svalyava" it was – 0.1 %.

An increase in daily diuresis is accompanied by an increase in the excretion of urea and creatinine from the body. Since most primary urine substances are reabsorbed in the convoluted tubules (and this process is energy-dependent), when the changes in reabsorption are the smallest, one could expect a more significant increase in the excretion of creatinine and urea; however, a considerable dependence is not determined.

Changes in urine pH were significant only in the case of the use of MW "Polyana Kupel". This indicator depends on the general activity of metabolism. Mg^{2+} is not determined in this MW; therefore, it can be assumed that the state of the general metabolism is such that it provides an additional intake of alkaline products into the urine.

The study of the effect of various MW with high H_3BO_3 content on the ion-exchange function of the kidneys established its unidirectional nature (Table 2). With the use of MW, a decrease in the concentration of ions in the urine was common while maintaining their excretion close to normal. At the same time, there were quantitative differences in the indicators of the state of the ion-exchange function of the kidneys with the use of various MW.

Since the decrease in the concentration of ions in the urine was more significant with the use of those MW that caused a tremendous increase in daily diuresis, it can be assumed that the decrease in the concentration of ions is due to an increase in urine volume, and not a violation of the excretion or reabsorption of ions.

Since the excretion of ions in the urine affects the ionic balance of the body, we evaluated the effect of MW on the electrolyte balance of the blood. According to the data in Table 3, the concentration of Na^+ in the blood of rats at the end of the course of MW use significantly increased (at the same time, its concentration in the urine significantly decreased). The content of K^+ in the blood also significantly increased against the background of a decrease in its content in the urine. Apparently, the reabsorption of K^+ increased since its excretion with urine does not change with the use of any of the investigated MW.

Table 2. Comparative assessment of the impact of MW high H_3BO_3 content on the ion-exchange function of the kidneys, M± m

Indicators	Groups			
	1st Group	2nd Group	3rd Group	4th Group
Concentration of K^+ in daily urine, mmol/l	126,59 ± 11,75	92,58 ±3,22*	80,30 ± 0,94*	79,10 ±3,98*
Daily excretion K^+, mmol	0,14 ± 0,017	0,14 ± 0,009	0,13 ± 0,002	0,13 ± 0,01
Concentration of Na^+ in urine, mmol/l	176,50 ± 6,67	136,37 ± 3,11*	148,90 ± 3,08*	154,60 ± 3,33*
Daily excretion of Na^+, mmol	0,19 ± 0,03	0,22 ± 0,01	0,23 ± 0,003	0,26 ± 0,02*
Concentration of CI^- in daily urine, mmol/l	297,07 ± 16,93	211,73 ± 6,40*	138,42 ± 5,86*	156,60± 10,68*
Daily excretion of CI^-, mmol	0,27 ± 0,02	0,30 ± 0,024	0,26 ± 0,007	0,28 ± 0,03

Note: M±m are arithmetic averages with errors of indicators; * - significant changes in indicators of 2nd, 3rd and 4th groups compared with the 1st group ($p<0.05$).

Table 3. The concentration of major ions in the blood of rats under the influence of MW with high H_3BO_3content, M ± m

Indicators	Groups			
	1st Group	2nd Group	3rd Group	4th Group
Concentration of Na^+ in blood, mmol/l	133,20 ± 0,68	138,58 ±0,74*	137,97 ± 0,15*	139,40 ± 0,35*
Concentration of K^+ in blood, mmol/l	4,47 ± 0,11	4,85 ± 0,09 *	5,20 ± 0,02*	5,46 ± 0,12*
Concentration of Ca^{2+} in blood, mmol/l	0,44 ± 0,03	0,61 ± 0,01*	0,59 ± 0,003*	0,62 ± 0,03*
Concentration of CI^- in blood, mmol/l	116,00 ± 0,18	108,04 ± 0,16*	112,04 ± 0,08*	109,00 ± 0,55*
Blood pH, c.u.	7,58 ± 0,01	7,57 ± 0,02	7,49 ± 0,01	7,63 ± 0,01

Note: M±m are arithmetic averages with errors of indicators; * - significant changes in indicators of 2nd, 3rd and 4th groups compared with the 1st group ($p<0.05$)

The concentration of Ca^{2+} in the blood plasma increased using the investigated MW. There was a quantitative difference in the changes in this indicator when using different MWs, but it did not correlate with the content of Ca^{2+} in the body. The content of CI^- in the blood decreased, but there was no significant difference in the changes in this indicator when using the “Polyana Ploskivska” and “Svalyava” MW. Blood pH did not change.

Histological studies of the kidneys of rats that used MW "Polyana Ploskivska", "Polyana Kupel", and "Svalyava" did not determine pathological changes in the structural and functional organization. It should be noted that in the presence of a general direction of changes in the structural and functional organization of the kidney tissue, there were some features of the reactions for each MW. Common to all MW was the uniform distribution of renal corpuscles, their rounded shape, and the expansion of Bowman's space. Features of reactions for MW "Polyana Ploskivska" – edema of endotheliocytes, the roundness of their nuclei, vakuoles in some endotheliocytes; for MW "Polyany Kupel" – edema of endotheliocytes, rounded nuclei, and pinching of part of the capillary glomeruli; for "Svalyava" – the presence of pawled capillary glomeruli. When applying each of the MW, damage to the convoluted tubules was not determined, but in all cases, there is swelling of the epithelium of the tubules. The interstitial layers in the kidneys in the application of all MW are thin, rather dense.

Histoenzymological studies have identified an increased activity of redox enzymes with the use of all MW. The SDH activity in epitheliocytes was - (7.00 ± 0.11) c. u.; LDH activity - (7.00 ± 0.08) c. u. In endotheliocytes, SDH activity was - (5.00 ± 0.12) c. u., LDH activity - (5.00 ± 0.12) c. u.

Thus, the research results prove that the structural and functional reactions of the urinary system when using MW with a different quantitative composition of trace elements and different H_3BO_3 content are similar but slightly differ in the quantitative parameters of the evaluated indicators. The volume of daily diuresis with the investigated MW increased from 27 to 45 %, and the percentage of tubular reabsorption decreased from 0.1 to 0,27%.

With the use of all MW, the excretory function of the kidneys increased, but the quantitative indicators were also different. The difference in the amount of excreted urea changed to the control within 33 % – 43%, creatinine -– from 18 % to 45 %.

It should be noted that changes in the percentage of tubular reabsorption were more extensive with the use of MW with a high content of H_3BO_3. Since the direct influence of the amount of H_3BO_3 on the reaction from the urinary system is not determined, it is legitimate to assume that the bioactive element of the MW determines the direction of the reactions of the kidneys to the use of the MW and the strength of the reaction is associated with the presence of microelements and the ratio of their amounts. This is supported by the similarity of changes in the structure of the kidney parenchyma when using MW with different quantities of H_3BO_3.

Analyzing the relationship between changes in the function of the kidneys and the characteristics of the macro composition of the studied MW, we determined the following. The most significant increase in diuresis was with the use of MW "Svalyava", in which, compared with other MW, the highest content of Ca^{2+} ,although the content (Na^{+} + K^{+}) was the lowest. In the MW, which caused an intermediate increase in diuresis, the content of (Na^{+} + K^{+}) was quite significant, and the content of Ca^{2+} was 16,8 times less than in the MW "Svalyava". That is, it is Ca^{2+} that contributes substuntially to the change in the function of urinary formation. In addition, it has been found that MW with the highest content of (Na^{+} + K^{+}) and H_3BO_3 causes the slightest changes in urination, urination, and ion exchange.

Thus, the nomenclature of the components of the macro composition, their ratio between themselves and the bioactive element, determines the difference in the reactions of the urinary system to the use of MW. Since the total number of ions in the MW plays a significant role in shaping the response of organs and systems, the osmolarity index can make it possible to predict the characteristics of the response of the urinary system to the use of any MW. In our case, the following was determined: the effective osmolarity of MW "Svalyava" was 132,2 mosmol/l (the lowest), and the diuretic and excretory effects were the greatest.

In MW "Polyana Ploskivska", the effective osmolarity is 254,4 mosmol/l (the highest), and the diuretic effect is the lowest. For “Polyana Kupel”, the osmolarity is intermediate, 224,7 mosmol/l, and its impact is medium. That is, the relationship between the MW′ osmolarity and the urinary system′ reaction force is determined.

CONCLUSIONS

Generally, it can be argued that the structural and functional response of the kidneys (its quantitative side) to the use of MW with a high content of H_3BO_3depends on the qualitative and quantitative composition of macro components. Osmolarity associated with the macro component composition can be used as a prognostic indicator.

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