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RESEARCH OF THE EFFECTIVENESS OF THE INFLUENCE OF MINERAL SILICON-SODIUM CHLORIDE WATER ON THE COURSE OF THE PATHOLOGY OF THE GASTROINTESTAL TRACT IN THE EXPERIMENT

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Abstract

In an experiment in white rats with a reproduced gastritis model, the effectiveness of the use of natural silicon salt sodium mineral water was investigated. Morphological studies at the end of the experiment macroscopically determined that the gastric mucosa is pink-brownish in color, the folds are visually somewhat coarse but of the usual height. Microscopically, the disappearance of manifestations of inflammation in the wall of the stomach was established. In the epithelial cells of the glands of the gastric mucosa, the activation of redox enzymes was established. The activity of redox enzymes (succinate dehydrogenase and lactate dehydrogenase) in the tissues of the stomach increases. The use of MW against the background of the reproduction of the gastritis model has a positive effect on the parameters of the peripheral blood and the immune system: a significant decrease in the percentage of neutrophils, restoration of the number of leukocytes and the hemoglobin content was established; restoration of the percentage of total T-lymphocytes, the range of circulating immune complexes and a significant decrease in the scope of antibodies to stomach tissue. The data obtained showed the feasibility of using silicon salt sodium mineral water in rats with experimental gastritis.

Keywords: *experimental gastritis, mineral water, structural condition of the stomach, hematological parameters*

Introduction

The pathogenesis of chronic gastritis (CG) is complex, multifactorial, and not fully understood, even though that this problem of modern medicine is given much attention [1, 2, 3]. Today, despite the success in treating chronic hepatitis, there is a frequent occurrence of complications, a tendency to relapse, and resistance to the applied therapy [4, 5]. Therefore, it becomes essential to develop non-drug technologies to treat chronic hepatitis, aimed at increasing the effectiveness of the treatment of this disease.

Based on the etiopathogenetic mechanisms of the development and progression of chronic hepatitis, it is advisable to add natural mineral waters (MW) to its complex treatment, which aims to reduce the recurrence of the disease and normalize the acid-forming function of the stomach. It is expected that this will increase the effectiveness of treatment of patients, reduce the dose of acid-suppressive drugs, prevent the progression of inflammatory-erosive processes in the esophagus, cancer prevention, and, thus, improve the quality of life of patients.

MW act on the digestive organs directly, mobilizing homeostatic systems from molecular to higher levels of biological integration and affecting the pathological process. MW also act "indirectly" through the gastroenteropancreatic endocrine system, affecting the endocrine, paracrine, and neuroendocrine regulation channels [6, 7, 8, 9].

So, the mechanism of action of drinking MW is associated not only with the accumulation of ions but with their effect on the endocrinocytes of the intestinal hormonal system, which forms urgent and long-term adaptive reactions that mediate the functioning reserves of both the regulatory blocks themselves and various organs and the whole organism as a whole [10, 11].

In this aspect, one should remember about sodium chloride MW. It is known that with the drinking use of chloride MW, the activity of metabolic processes increases, a choleretic effect appears. Prolonged use contribute to the rise in the secretion of gastric juice. When taking MW, sodium ions have a pronounced stimulating effect on the secretory apparatus of the digestive tract [12]. MW containing sodium and chlorine ions have a stimulating effect on the secretion of hormones of the pituitary gland and adrenal cortex, which are the main regulators of the general resistance of the body, that is, resistance to the action of factors of a very different nature (alcohol, high-altitude hypoxia, radiation, etc.) [13, 14].

That is, the positive effect of the use of MW is associated with the peculiarities of their physicochemical composition, and at the same time, with the presence of not only macroelements but also microelements (iron, vanadium, arsenic, bromine, iodine, boron, fluorine, chromium, silicon, etc.) and biologically active components and compounds (hydrogen sulfide, carbon dioxide, radon, organic substances, etc.), and what is essential - the ratio of macro-microelements and biologically active components [14, 15, 16]. In addition, in the composition of MW, ions and biologically active ingredients and compounds are in the most active form for assimilation, which determines their significant biological effect [17, 18, 19].

Today, one of the most studied trace elements is silicon. It is indicated that this trace element takes part in the synthesis of glycosaminoglycans, elastin, and collagen, which form the skeleton of the connective tissue and give it strength and elasticity, strengthens the walls of blood vessels, which is necessary for the formation of the primary substance of bone and cartilage, etc. [20, 21]. In epithelial tissues, silicon is in the form of low molecular weight compounds of cell membranes, the presence of which determines their elasticity and impermeability [22].

Considering its prevalence both in nature and in the body of mammals, several authors note that silicon is essential for ensuring the health of humans and animals while paying attention to its active participation in the restoration of cell membranes [23, 24, 25]. Studies on the effect of silicon chloride sodium MW of chemical composition in experimental damage to the gastric mucosa are few and not systematized [26].

The work aimed was to evaluate the effectiveness of the use of silicon sodium hydroxide water in rats with a gastritis model.

Methods

The studies were carried out on 34 white female Wistar rats of outbred breeding, with a bodyweight of 180.0 - 200.0 g (the animals were obtained from the vivarium-nursery of the private enterprise "Biomodelservice" of the State Institution "Institute of Pharmacology and Toxicology of the Academy of Sciences of Ukraine", Kyiv). The animals were kept in standard drinking and light conditions in the vivarium of the State Institution "Ukrainian Research Institute of Medical Rehabilitation and Resort Therapy of the Ministry of Health of Ukraine", Odessa, Ukraine. The studies were carried out following existing guidelines and legal documents [27, 28, 29].

The animals were divided into three groups. The first group (10 pcs.) - intact animals that consumed settled tap water in the mode of free access to drinking troughs were not exposed to any external influences and were used as a control group. The second group (12 pcs.) - rats with a gastritis model. The third group (12 pcs.) - rats with a gastritis model, which received MW.

The gastritis model was induced by injecting a potassium permanganate solution into the rat's stomach. For this purpose, for two days in a row, a solution of potassium permanganate at a dose of 200 mg per 1 kg was injected into the animal's stomach with a soft probe with a metal olive. For rats weighing 200.0 g, the solution for administration was prepared as follows: 40 mg of potassium permanganate was dissolved in 2 ml of distilled water [30]. The choice of this model is due to the fibrosing effect of potassium permanganate on the vessels of the stomach wall.

Starting from the 3rd to the 9th day of the experiment, the rats of the 3rd group received MW internally. Rats received MW orally, through a soft probe with an olive, once a day at a dose of 1% of the animal's body weight. The animals were withdrawn from the experiment under ether anesthesia by the decapitation method. Macro and microscopic assessment of the structural and functional organization of stomach tissues was carried out, for which two pieces of the organ were removed from rats.

The first piece was passed through alcohols of increasing concentration and poured into celloidin. Histological sections were prepared and stained with hematoxylin-eosin. The obtained sections were used for microscopic studies of structural changes in the kidneys. The second piece was frozen with dry carbon dioxide (- 70° C), on the prepared cryostat sections, histochemical reactions were carried out to determine the activity of succinate dehydrogenase (LDH) and lactate dehydrogenase (LDH) according to Loyda's recipe, the enzyme activity was evaluated in conventional units of optical density [31].

Based on hematological studies, the reaction from the peripheral blood to the development of experimental gastritis was determined, which was assessed by changes in the number of leukocytes, the ratio of the elements of the blood formula. The assessment of the state of the immune system was evaluated by changes in its cellular and humoral links. The response from the cellular link of the immune defense was assessed by determining the number of total T-lymphocytes and their subpopulations: T-helpers and T-suppressors).

The activity of the phagocytic process was assessed by determining the number of active phagocytes, their absorbing function - phagocytic index (PI), a metabolic function in the nitro blue tetrazolized test - NBT test (spontaneous and stimulated). The response from the humoral link of the immune defense was assessed by determining the content of antibodies to stomach tissues and the range of circulating immune complexes (CIC). All methods used were approved by the Ministry of Health of Ukraine [32].

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). 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 study used mineral waters from well No. 114, which is located on the territory of the health-improving complex "Belaya Akatsia" in Odessa, Ukraine. According to the legislation of Ukraine, this water is characterized as medium-mineralized sodium chloride with a high content of biologically

active substances - silicon [33]. Silicon content in the form of metasilicic acid ranges from 68.15 mg / l to 78.58 mg / l), with a balneological norm of 50.0 mg / l. Total mineralization of water ranges from 5.56 g / l to 6.20 g / l. The content of chloride ions varies from 2907.3 mg / l to 3309.6 mg / l sodium and potassium ions - from 1843.2 mg / l to 2097.8 mg / l.

Results

Morphological studies of rats with experimental gastritis have established that on the 3rd day of the experiment, the mucous membrane is thickened, coarse, folds are enlarged, coarse. The mucous membrane is reddish-brown. On the fifth day of the experiment, it did not change macroscopically. On the seventh day of gastritis modeling, the folds approach their usual height, the color of the mucous membrane becomes pale, the mucous membrane becomes visually coarse. At the end of the experiment (the ninth day) - the mucous membrane is pale brown, the folds of the mucous membrane are reduced, its coarseness remains.

Microscopically, on the 3rd - 5th day of the experiment, the submucosa consists of swollen, densely packed fibers, infiltrated with juicy lymphoid elements. The mucous membrane is thickened due to an increase in the diameter of the tubular glands and edema of the fibers of the interstitial layers. There is a massive diffuse infiltration with lymphoid elements. The epithelial cells of the glands are enlarged, their cytoplasm is edematous, light-colored, the nuclei are medium in size, juicy. In the excretory ducts, the size of the goblet cells, which are overflowing with mucus, is sharply increased.

On the seventh day of gastritis modeling, the fibers are tightly packed, swollen, diffuse infiltration by lymphocytes remains in the submucosa. In the mucous membrane of the gland of a tubular form of increased diameter, the location of epithelial cells is disordered; due to this, the lumen of the glands is not readable. Their cytoplasm is light-colored, a granular-fibrous pattern is visible in the nuclei, the interstitial layers are thin, their fibers are somewhat coarse. In the mucosa, there is a diffuse rather massive lymphoid infiltration; the vessels are partially spasmodic.

On the ninth day of the experiment, along with edema of the fibers and moderate lymphoid infiltration, vascular spasm and fibrosis of their walls are observed in the submucosa. In the mucosa, the width of which is regular, in the interstitial layers, the fibrous fibers remain in an altered form, there is a moderate lymphoid infiltration. The location of epithelial cells in most glands is ordered; their cytoplasm is weakly basophilic, the nuclei are medium in size, located closer to the basement membrane. The goblet cells of the excretory ducts are large enough, filled with mucus.

SDH activity during the experiment - (5.0 ± 0.20) c. u., but there are fields in which the activity of SDH in epithelial cells is (6.0 ± 0.11) c. u. The activity of SDH in epithelial cells at the end of the experiment was (7.0 ± 0.30) c. u; LDH activity in epithelial cells close to the surface - (5.0 ± 0.21) c. u., and closer to the submucosa - (6.0 ± 0.29) c. u. The LDH activity of epithelial cells at the end of the experiment was (7.0 ± 0.30) c. u. That is, in the epithelial cells of the glands of the gastric mucosa, there is an activation of the enzymes of anaerobic glycolysis.

The data of hematological parameters are shown in Table 1. The state of the peripheral blood of rats of group 2 with a gastritis model is characterized by an increase in the total number of leukocytes by 13% ($p < 0.001$), redistribution of blood cells - the percentage of neutrophils increased by 48%, and lymphocytes decreased by 8% ($p < 0.001$). Also, the hemoglobin level significantly reduced by 8% ($p < 0.01$). Other indicators - the ESR value, the number of monocytes, eosinophils, erythrocytes, and the color indicator did not differ from the 1st group of intact animals ($p > 0.5$).

The study of the state of the immune system in rats of group 2 revealed signs of suppression of the cellular component of the immune defense (Table 1). The number of total T-lymphocytes decreased by 25% ($p < 0.001$) compared with the control group due to a significant decrease in the number of both T-helpers and T-suppressors ($p < 0.001$) while maintaining the immune regulatory index within the normal range ($p > 0.5$), which indicates transient changes in the cellular link of immunity in rats with a gastritis model. On the part of the indicators of phagocytic processes, only a decrease in the absorptive capacity of active phagocytes of peripheral blood was observed while maintaining their number and metabolic function within the normal range (indicators of spontaneous and stimulated NBT test). On the part of the indicators

of the humoral link of the immune response, there is a slight but significant increase in the CIC content ($p < 0.05$) and a significant increase in the content of antibodies to stomach tissue by 140% ($p < 0.01$), which indicates the development of a pathological process.

In rats of group 3 with a gastritis model, which received MW, morphological studies were carried out once - after the end of the experiment. On macroscopic examination, the gastric mucosa is pink-brownish in color, the folds are visually somewhat coarse but of the usual height. Microscopically - in the stomach, the submucosa is dense, the nuclei of fibroblasts are juicy colored. There is no lymphoid infiltration. The glands of the mucous membrane are of the usual tubular shape; the lumens are dilated, the cytoplasm of the epithelial cells is homogeneous, the nuclei are juicy colored. In the layers of the interstitium, there are single lymphocytes; the layers themselves are somewhat expanded, the vessels are primarily of moderate blood filling. SDH activity in epithelial cells - (7.00 ± 0.21) c. u.; LDH activity - (7.00 ± 0.10) c. u. That is, the activity of redox enzymes is increased.

We have established a positive effect of MW on the peripheral blood parameters of rats with a gastritis model (Table 1.). The leukocyte count and hemoglobin content reached the level of intact animals. There was a tendency to an increase in the number of erythrocytes.

Course internal use of MW in rats with a gastritis model has a normalizing effect on the immune system. It was established that the subpopulation of T-helpers and T-suppressors lymphocytes was restored to the level of intact animals. The percentage of total T-lymphocytes also significantly increased and significantly differed from this indicator in animals from group 2 ($p < 0.01$) but did not reach the level of intact animals. On the part of the indicators of the humoral link of the immune defense, a decrease to the level of reference values of the CIC content and a reduction in the content of antibodies to stomach tissue from 140% to 64% ($p < 0.01$) were determined, which indicates a limitation of the development of inflammatory processes.

Conclusions

According to the results of the morphological studies, it was found that the use of MW promotes the restoration of structural elements in the stomach in experimental gastritis. The height of the folds decreases, the blood filling of the vessels improves, the ordering of epithelial cells in the glands of the mucous membrane is restored, the lymphoid infiltration of the gastric mucosa disappears. In the tissue of the stomach, the activity of redox enzymes increases. In the study of hematological parameters, the disappearance of leukocytosis, a decrease in signs of neutrophilia and lymphopenia, restoration of red blood parameters (hemoglobin and erythrocyte content), normalization of the parameters of the cellular and humoral links of the immune system were established. The established positive changes indicate that silicon hydrochloric sodium MW in rats with experimental gastritis promotes recovery processes.

Based on the data presented, it is possible to recommend using of silicon hydrochloric sodium MW in the complex treatment of patients with gastritis.

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Table 1. Hematological parameters of rats with gastritis and rats with gastritis, internally receiving a course of MW

Indexes	Ist group	IInd group	IIIrd group
	($M_1 \pm m_1$)	($M_2 \pm m_2$)	($M_3 \pm m_3$)
Leukocytes, 10^9/l	5,50 ± 0,26	6,24 ± 0,30*	5,57 ± 0,19
ESR, mm/h	1,60 ± 0,13	1,50 ± 0,22	1,61 ± 0,12
Neutrophils,%	12,80 ± 0,60	19,07 ± 0,93*	16,58± 0,61*
Eosinophils,%	2,32 ± 0,26	2,85 ± 0,68	2,70 ± 0,23
Monocytes,%	3,75 ± 0,24	3,48 ± 0,27	3,29 ± 0,21
Lymphocytes,%	81,2 ± 0,80	74,8 ± 1,24*	77,7 ± 0,73*
Hemoglobin, g / l	157,08±4,71	144,40± 1,81*	155,67 ± 0,88
Erythrocytes, 10^{12} / l	4,01 ± 0,09	3,79 ± 0,13	4,23 ± 0,04
Color indicator, c.u.	1,09 ± 0,01	1,15 ± 0,02	1,11 ± 0,02
T-lymphocytes, %	47,22 ± 0,6	36,0 ± 1,30*	43,0 ± 0,85
T-helpers, %	29,33 ± 1,12	24,0 ± 1,46*	29,28 ± 1,62
T-suppressors, %	17,6 ± 0,80	11,6 ± 0,73*	13,7 ± 1,71*
T-helpers / T-suppressors, c.u.	1,66 ± 0,11	2,07 ± 0,22	2,14 ± 0,35
Phagocytosis, the number of active phagocytes, %	39,9 ± 0,53	39,2 ± 0,44	39,6 ± 0,57
Phagocytic index	2,10 ± 0,10	1,80 ± 0,04*	1,95 ± 0,03
HCT test, mg: spontaneous stimulated	 0,039 ± 0,001 0,090 ± 0,002	 0,039 ± 0,002 0,091 ± 0,001	 0,039 ± 0,001 0,089 ± 0,001
Antibodies to gastric tissues, u.c.	5,07 ± 1,47	12,05 ± 1,43*	8,36 ± 1,70*
CIC, mg/ml	5,70 ± 0,11	6,18 ± 0,15*	5,64 ± 0,20

Notes. ($M_1 \pm m_1$), ($M_2 \pm m_2$) and ($M_3 \pm m_3$) are arithmetic means with errors;

*- $p < 0.05$