BIOLOGICAL RESEARCH

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PECULIARITIES OF SUCTION, EXTRACTION AND ASSIGNMENT OF CALCIUM IN EXPERIMENTAL CHRONIC HEPATITIS IN RATS

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The aim. *Study the degree of absorption, assimilation, and excretion of calcium in rats with chronic toxic hepatitis.*
Materials and methods. *The studies were carried out on 1 month old Wistar rats. Toxic hepatitis in the experimental group was reproduced by intraperitoneal injection of hydrazine sulfate at a dose of 50 mg / kg twice a week. Studies of the absorption of substances in rats were carried out under thiopental anesthesia according to the Tyri method after 3 months of pathology modelling. In the intestinal contents, the amount of unabsorbed calcium and amino acids was determined. To determine the amount of assimilated and excreted calcium metabolic chambers were used for daily collection of urine, faeces and food residues, in which the calcium content was determined (average for three days per animal). After removing the rats from the experiment, the level of calcium in the blood serum was determined, in the bone tissue – the degree of its resorption by the activity of acid phosphatase and the content of calcium.*
Results and discussion*. Toxic hepatitis reduced calcium absorption by 34.5 % in the small intestine of rats and did not have a significant effect on amino acids, the inhibition of absorption of which in hepatitis was only 5.5 %. The excretion of calcium in the urine of rats with toxic hepatitis was reduced by 1.8 times, and with faeces, on the contrary, increased by 1.5 times. As a result, calcium absorption in rats with hepatitis decreased by 24.2 %. Decreased absorption of calcium, and its increased excretion in the faeces, led to a decrease in the level of this element in the blood of animals with hepatitis by 14.7 %. Our studies found bone destruction in rats with hepatitis: an increase in bone acid phosphatase activity by 65.3 % and a decrease in calcium levels by 15.5 %.*
Conclusion*. The triggering mechanism for the development of hepatic osteodystrophy is the inhibition of calcium absorption in the small intestine of rats with hepatitis, consequently - a decrease in its absorption and level in the blood, which ultimately leads to the activation of bone resorption. The established patterns will form the basis of the pathogenetic scheme for the prevention of hepatic osteodystrophy*
Keywords: *toxic hepatitis, hydrazine sulfate, calcium, small intestine, amino acids, hepatic osteodystrophy*

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1. Introduction

Recently, there has been increasing attention in the world to the problems associated with chronic liver diseases. There is a significant spread of pathologies associated with liver damage, in particular chronic hepatitis. A proven fact is the relationship between impaired liver function and changes in bone tissue, which is commonly called hepatic osteodystrophy [1]. It is known that liver pathology is accompanied by a decrease in calcium absorption in the small intestine due to impaired hydroxylation and the formation of 25-hydroxyvitamin D [2, 3]. We have previously established that long-term administration of hydrazine sulfate to laboratory rats, in addition to pathological changes in liver function, leads to a violation of the structure and remodelling processes in the bone tissue of the jaws and femurs of animals [4].

It has been suggested that a possible trigger for the development of osteodystrophy in diseases of the liver and biliary tract may be inflammatory and dysbiotic processes in the mucous membrane of the small intestine. Disturbances in the composition of the intestinal microbiota occur in most patients with chronic hepatitis and cholelithiasis [5]. Our previous studies have also established the development of inflammatory and dysbiotic processes in the mucosa of the small intestine of laboratory rats with hepatitis, which can cause malabsorption of substances necessary for building bone tissue and, as a result, the development of osteodystrophy [6].

Therefore, the purpose of this fragment of the work was to study the degree of absorption of calcium and amino acids in the small intestine and clarify the na-

ture of absorption and excretion of calcium in rats with chronic toxic hepatitis.

2. Materials and methods

The research was conducted based on the vivarium of the Faculty of Biology of the I. I. Mechnikov Odessa National University and the Laboratory of Biochemistry the Institute of Stomatology and Maxillo-Facial Surgery National Academy of Medical Science of Ukraine during the summer of 2021.

The studies were carried out on 1 month old Wistar rats of herd breeding. Toxic hepatitis in the experimental group was reproduced by intraperitoneal injection of hydrazine sulfate at a dose of 50 mg / kg twice a week.

Studies of the absorption of substances in rats were carried out under thiopental anesthesia (20 mg/ kg) [7]. 1.5 ml of an ionized calcium solution or a mixture of aromatic amino acids (phenylalanine, tyrosine, tryptophan) was injected into an isolated section of the small intestine 5 cm long. After 40 minutes, in the solution that remained after absorption, the concentration of calcium and amino acids was determined, having previously measured the volume of the liquid obtained. The number of substances was determined by multiplying the volume by the concentration. The content of ionized calcium in the intestinal contents was determined by direct potentiometry, and the number of amino acids was determined by reaction with Folin's reagent [8].

To study the intensity of excretion and assimilation of calcium, experimental animals were placed in metabolic chambers for the daily collection of urine, faeces and food residues, in which the calcium content was determined (average for three days per animal) [9].

Animals were taken out of the experiment by total bloodletting from the heart under thiopental anesthesia. Calcium levels were determined in the blood serum of rats. In homogenates of the jawbone tissue (75 mg/ml 0.1 M citrate buffer pH 6.1), the activity of acid phosphatase (resorption marker) and the calcium content [9].

The maintenance and removal of animals from the experiment was carried out in accordance with the provisions established by the Directive of the European Parliament and Council (2010/63/EU) and the order of the Ministry of Education and Science, Youth and Sports of Ukraine dated 01.03.2012. No. 249 [10, 11].

Statistical processing of data in the study was carried out according to the Student-Fisher's method. Significant deviations were considered those that were within the probability limits according to Student's tables, $p \leq 0.05$. Statistical processing of the study results was carried out using the standard Microsoft Excel software product and the Statistika package.

3. Results

Table 1 shows the results of a study of the absorption of calcium and a mixture of aromatic amino acids in the small intestine of experimental animals. Chronic toxic hepatitis caused by hydrazine sulfate contributed to a significant decrease in calcium absorption by 34.5 % ($p<0.001$) in the small intestine and did not significantly affect the absorption of amino acids, since inhibition of amino acid absorption was only 5.5 % ($p>0.5$, Table 1).

Table 1

Absorption of calcium and amino acids in the small intestine of rats after prolonged administration of hydrazine sulfate (M±m; n=20)

Groups of animals	Amount of injected substance	Residual quantity	Amount of absorbed substance
Calcium, mmol			
Intact	15.13±0.53	3.64±0.19	11.50±0.24
Hydrazine sulfate	15.13±0.41 p>0.9	7.60±0.23 p<0.001	7.53±0.17 p<0.001
Amino acids (phenylalanine, tyrosine, tryptophan), μmol			
Intact	2.83±0.17	0.73±0.06	2.10±0.15
Hydrazine sulfate	2.83±0.17 p>0.9	0.84±0.09 p>0.5	1.99±0.12 p>0.5

Note: p – is the significance of differences in indicators in comparison with the intact group

We hypothesize that a decrease in calcium absorption may be the result of the development of inflammation and dysbiosis in the mucous membrane of the small intestine of growing rats under the influence of long-term administration of hydrazine sulfate, which was established by us in earlier studies [6].

Long-term administration of hydrazine led to a violation of the excretion and absorption of calcium in rats with hepatitis, as evidenced by Table 2, which presents the results of determining calcium in daily urine, faeces and in the food eaten (average over 3 days). Thus, intact rats with food received calcium by 16.5 % (although $p>0.5$) more than animals with hepatitis. This is due to the consumption of less food in hepatitis, probably due to general intoxication and the development of hyporexia. Urinary calcium excretion in rats with toxic hepatitis was significantly reduced by 1.8 times ($p<0.001$), and in faeces, on the contrary, increased by 1.5 times ($p<0.01$).

As a result, the total amount of excreted calcium in the urine and faeces in animals with hepatitis did not change significantly ($p>0.5$).

However, summarizing all the data obtained, the final absorption of calcium in rats with hepatitis decreased by 24.2 % ($p<0.001$, Table 2).

Studies of the calcium level in the blood revealed its significant decrease by 14.7 % ($p<0.001$) in rats with chronic hepatitis, which is a consequence of a decrease in its absorption in the small intestine mucosa and led to inhibition of excretion by the kidneys (Table 3).

Table 2

Excretion and absorption of calcium in rats with chronic toxic hepatitis, mg (M±m; n=20)

Indicators	The control	Hepatitis
Feed intake, mg	12.92±0.62	10.79±0.40 p>0.5
Excretion with urine, mg	1.62±0.06	0.90±0.005 p<0.001
Excretion with faeces, mg	1.89±0.17	2.76±0.17 p<0.01
Total excretion, mg	3.51±0.20	3.65±0.22 p>0.5
Absorption, mg	9.41±0.09	7.13±0.14 p<0.001

Note. p – the significance of differences in indicators in comparison with the intact group

Table 3

The content of calcium in the blood serum and bone tissue of rats after prolonged administration of hydrazine sulfate (M±m; n=20)

Groups of animals	Serum calcium content, mmol/l	The content of calcium in bone tissue, mmol/kg	Acid phosphatase activity, mk-kat/kg
Intact	2.24±0.10	3.23±0.39	2.75±0.17
Hepatitis	1.91±0.05 p<0.001	2.73±0.19 p<0.1	4.48±0.53 p<0.05

Note: p is the significance of differences in indicators in comparison with the intact group

4. Discussion of research results

A compensatory response to insufficient calcium absorption in the small intestine and its deficiency in the blood, as you know, is an increase in bone resorption, which serves as a calcium depot, under the influence of parathyroid hormone. This was confirmed by our studies, which found an increase in the marker of resorption – the activity of bone acid phosphatase by 62.9 % ($p<0.05$) in the jaws of rats with toxic hepatitis. In the bone tissue of rats that were injected with hydrazine sulfate for a long time, there was also a decrease in the level of calcium by 15.5 % (although $p<0.1$).

Thus, long-term administration of hydrazine sulfate to laboratory rats contributed to a decrease in calcium absorption in the small intestine and a decrease in its excretion in the urine. Calcium, which was not absorbed into the blood in the small intestine and was not absorbed, was excreted in the faeces. The resulting calcium deficiency in the blood of animals with hepatitis compensatory induced bone tissue resorption and, as a result, the development of osteodystrophy.

In turn, the cause of impaired absorption and assimilation of calcium in toxic hepatitis may be the development of dysbiosis due to liver pathology, which has been proven by us [6] and other authors.

So, a violation of the bile-forming function of the liver causes a deficiency of bile acids, helps to reduce the bactericidal function of bile, and provokes excessive bacterial growth in the intestine, disrupts lipid hydrolysis, slows down intestinal motility and the biliary system [12]. Research by Seliverstov P. V. with the authors found that dysbiosis in the small intestine reduces the content and activity of intraluminal and parietal enzymes, disrupts the processes of digestion and absorption.

These processes are the result of a drop in the intra-intestinal pH level, destruction of enzymes by microbites, and the development of structural disturbances in the brush border of enterocytes during adhesion of opportunistic bacteria to the intestinal mucosa [12].

On the other hand, since the absorption of amino acids in the small intestine in rats with toxic hepatitis did not change significantly, the decrease in the degree of calcium absorption, most likely, may be the result of impaired absorption of vitamin D, also induced by dysbiosis – contamination of the intestine with opportunistic and pathogenic microbiota in the presence of pathology liver. Confirmation is the data on malabsorption of fat-soluble vitamins A, D, E, K and calcium in patients with chronic liver diseases [13, 14].

In addition, the violation of calcium absorption in the small intestine of rats with hepatitis, which we found, may be a consequence of inhibition of the synthesis of the calcium carrier protein (α1-globulin), which is carried out in the liver [15].

Summarizing our results and data from other studies, we can conclude that the triggering mechanism of osteodystrophy in liver pathology is a violation of the absorption of calcium and fat-soluble vitamins, including D, mediated by the development of dysbiosis and inflammation in the mucous membrane of the small intestine. Along with dysbiosis, a known factor that reduces the absorption and assimilation of calcium in liver failure is a direct violation of the function of hepatocytes, in particular, inhibition of the synthesis of α_1-globulin, which is involved in the transfer of calcium through the enterocytes of the small intestine mucosa into the blood, as well as a violation of vitamin hydroxylation D and the formation of 25-hydroxyvitamin D (calcifediol).

The results of the study are a pathogenetic rationale for the inclusion in the scheme of prevention of hepatic osteodystrophy of drugs that prevent the development of dysbiosis in chronic liver pathologies.

The limitations of the study are the result of an experimental model of chronic hepatitis, which allows us

to assess the impact of pathology on calcium metabolism and bone status but ignores the possible impact on other components of the disease.

Prospects for further research. Research and obtaining new data on the pathogenetic resorption of bone tissue under the influence of chronic liver disease are promising. Based on this, a scheme of preventive measures will be developed.

5. Conclusions

1. Modelling of chronic toxic hepatitis using hydrazine sulfate contributed to a decrease in the absorption of calcium by 34.5 % and aromatic amino acids by 5.5 % in the small intestine of rats.

2. Rats with hepatitis received 16.5 % less calcium with food than control animals. Calcium excretion with urine in rats with hepatitis was reduced by 1.8 times, and with faeces – increased by 1.5 times. As a result, the final absorption of calcium in rats with hepatitis decreased by 24.2 %.

3. In rats with chronic hepatitis, the calcium content in the blood serum decreased by 14.7 %, in the bone tissue of the jaws – by 15.5 % against the background of an increase in the activity of bone acid phosphatase by 62.9 %.

Conflict of interest

The authors declare that they have no conflicts of interest.

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