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ORIGINAL ARTICLE

EVALUATION OF MEMBRANE-DESTRUCTIVE PROCESSES IN RATS WITH INDUCED CARCINOGENESIS OF THE COLON USING THE CITOSTATIC VINCRISTINE

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ABSTRACT

Background. Every year the number of cases of colorectal cancer increases. Chemotherapy is one of the main methods of treating cancer. However, chemotherapeutic treatment of colorectal cancer is inextricably linked to hepatotoxic reactions.
Objective. The aim of this study was to investigate the effect of the cytostatic vincristine on the background of previous enterosorption correction with the drug aut-m in adenocarcinoma of the colon.
Material and methods. To simulate carcinogenesis, dimethylhydrazine (DMH) was administered subcutaneously to 77 rats for 30 weeks at a dose of 7.2 mg/kg body weight. After simulation of colon cancer, the animals were intragastricly administered entorosorbent at a dose of 1 ml of suspension (corresponding to 0.2 g of net weight of the drug) per 100 g of body weight of the animal, daily for 21 days. After detoxification therapy, rats with simulated carcinogenesis were administered the daily cytostatic vincristine at a dose of 0.23 mg/kg for 14 days.
Results. It was found that prolonged administration of dimethylhydrazine is accompanied by destructive changes in plasma membranes, as evidenced by increased activity of enzymes alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and serum urea.
Conclusions. The used sorbent aut-m showed an effective effect on reducing the manifestations of cytolytic processes in induced carcinogenesis, as indicated by the normalization of the studied parameters. The cytostatic vincristine, which was used in rats with induced colorectal cancer after enterosorption therapy, did not significantly affect the enhancement of cytolytic processes, which confirms the effectiveness of previous sorption measures under these conditions.

Key words: *dimethylhydrazine, colorectal cancer, membrane destructive processes, enterosorbent aut-m*

INTRODUCTION

Colorectal cancer (CRC) is one of the leading causes of cancer deaths in the world, and its incidence is rising in developing countries. According to the International Agency for Research on Cancer, the number of cancer patients worldwide in 2018 was over 18 million. More than 600,000 cases of CRC are reported worldwide each year, and less than a third of these patients live for more than 5 years [16].

One of the main methods in the treatment of cancer is chemotherapy. However, toxic side effects of chemotherapy components can sometimes lead to discontinuation of treatment before a clear antitumor effect is obtained [8]. Chemotherapy for cancer in some patients is accompanied by a hepatotoxic reaction, which may progress both during treatment and in between courses or after their completion. Violation of the integrity of plasma membranes of hepatocytes indicates the development of fatty degeneration, hepatocellular necrosis and fibrosis, the appearance of duct disorders with cholestasis, changes in the activities of aminotransferases, alkaline phosphatase [3]. Liver damage as a side effect can be observed with the use of almost all groups of cytostatics.

Finding effective remedies to alleviate the side effects of anticancer drugs is an extremely important problem in medicine [8]. There is information in the literature on the effectiveness of the use of sorbents in the therapeutic support of cancer patients. Analyzing the results of many years of study of the mechanisms of action of enterosorbents, we can conclude that

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the fibrous, carbon sorbent AUT-M deserves special attention [13, 14]. The drug itself consists of micro-, meso- and macropores and has a specific pore surface area of about 2000-2500 m/g, which allows to absorb a wide range of substances of different molecular weight [9,10].

The aim of this work was to study the effect of the cytostatic Vincristine on the background of previously performed enterosorption correction with the drug AUT-M in adenocarcinoma of the colon.

MATERIALS AND METHODS

Materials

Studies were performed on 77 white male rats weighing 200–250 g. Laboratory animals were kept on a standard diet of the vivarium of Horbachevsky Ternopil National Medical University in accordance with the rules of the European Convention for the Protection of Vertebrate Animals Used for Scientific and Other Scientific Purposes. The study was approved by the Ethics Commission of the Horbachevsky Ternopil National Medical University Gorbachevsky (Excerpts from the protocol №61 from 13.11.2020).

Scheme of experimental research

Adenocarcinoma of the colon in rats was simulated by subcutaneous administration of the carcinogen 1,2-dimethylhydrazine (DMH) (Sigma-Aldrich Chemie, Japan), pre-diluted with saline in the interscapular region at a dose of 7.2 mg / kg [2, 5, 6].

DMH was administered once a week for 7 months. After modeling the cancer process, the animals underwent detoxification correction with the sorbent AUT-M. The sorbent was administered intragastric daily for 21 days. The daily dose of sorbent was 1 ml of suspension (corresponding to 0.2 g of net weight of the drug) per 100 g of body weight of the animal.

The next step was cytostatic correction with Vincristine. Vincristine was administered for 14 days, intragastric at a dose of 0.23 mg/kg daily, the dose was recalculated according to *Rybolovlev*, taking into account species sensitivity [12].

Rats were divided into three groups: I - control animals, which were injected with saline subcutaneously in the interscapular area once a week for 30 weeks; II - animals with simulation of adenocarcinoma of the colon; III - animals with imitation of adenocarcinoma of the colon and 21-day extracorporeal detoxification with sorbent AUT-M; IV - animals with simulated carcinogenesis of the colon, which after 21 days of entorosorption correction were administered for 14 days cytostatic Vincristine.

Animals were removed from the experiment once a month for 7 months, on the 14th and 21st day of administration of the enterosorbent AUT-M, as well as on the 14th day of administration of the cytostatic Vincristine. All manipulations were performed under thiopental anesthesia.

Methods

Blood and liver samples were used in subsequent studies. Blood was taken from the hearts of animals. To obtain serum, blood samples were allowed to coagulate (at room temperature for 30 min), then centrifuged for 15 min at 1200 g and room temperature. To prepare 10% liver homogenate, samples taken immediately after euthanasia were cooled to 1-3 ° C in saline, dried on filter paper and homogenized in 0.05 M Tris-HCl buffer (pH 7.4) using a SilentCrusher S magnetic homogenizer (Haydolp, Germany).

The intensity of membrane-destructive processes in the liver was assessed in serum and liver homogenate by the activity of enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and urea content [19]. Enzyme activity was determined by turbidimetric method using a semi-automatic biochemical analyzer Humalyzer 2000 using a colorimetric set of reagents Human (Germany).

Statistical analysis of the data was performed using STATISTICA 13 (TIBCO Software Inc., 2018). Parametric and non-parametric methods of evaluation of the obtained data were used for statistical processing of the results. The arithmetic mean of the sample (M) and the error of the arithmetic mean (m) were calculated for all indices. The reliability of the difference between the values between the independent quantitative values was determined by the normal distribution using *Student's* t-test, in other cases by the *Mann-Whitney* test. The difference between the values was considered probable at $p < 0.05$.

RESULTS

It is known that the development of a malignant process is accompanied by impaired liver function, in particular, changes in the permeability and structure of hepatocytes [5, 6].

Changes in the activity of membrane-dependent enzymes, such as aminotransferases, have been revealed in the dynamics of modeling DMH-induced carcinogenesis. Thus, after 1 month of DMH administration, ALT activity in the serum of affected rats probably ($p < 0.05$) increased by 36%, on the 4th month by 56% compared with the activity of this enzyme in animals of the control group (Figure 1). In the following terms of the study there was a slight decrease in ALT activity compared to previous terms (5 months - the activity of the enzyme was only 42% higher than the level of control animals, 7 months - 25% higher than their level).

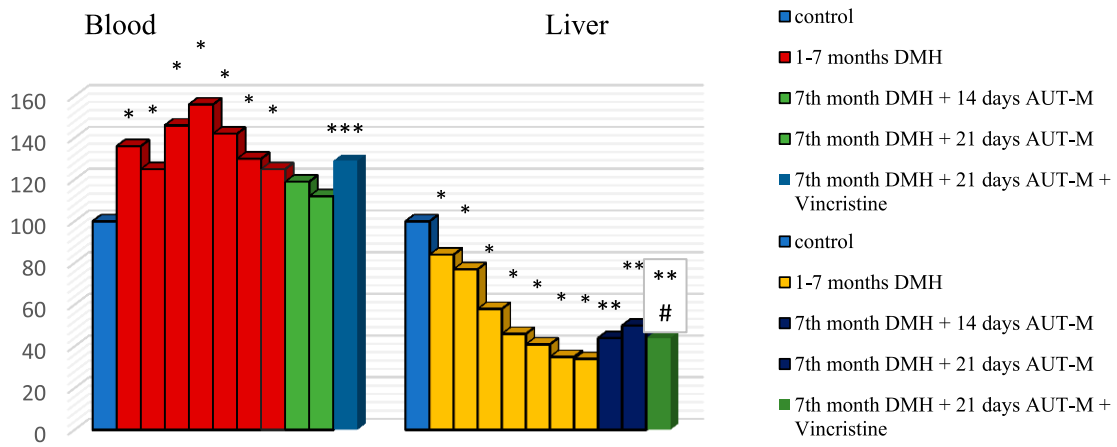

Figure 1. Dynamics of changes in ALT activity in the serum and liver of rats with induced carcinogenesis after the use of cytostatics on the background of previous detoxification

Note: here and in the following figures * - probable changes between the indicators of the animals of the control group and those affected by DMH; ** - probable changes between the rates of carcinogenic animals and animals that received enterosorbent; *** - probable changes between the rates of carcinogenic animals after enterosorption therapy (21 days) and animals receiving cytostatics (14 days); # - probable changes between carcinogenic animals (7 months) and animals receiving cytostatics (14 days) on the background of enterosorption therapy (21 days)

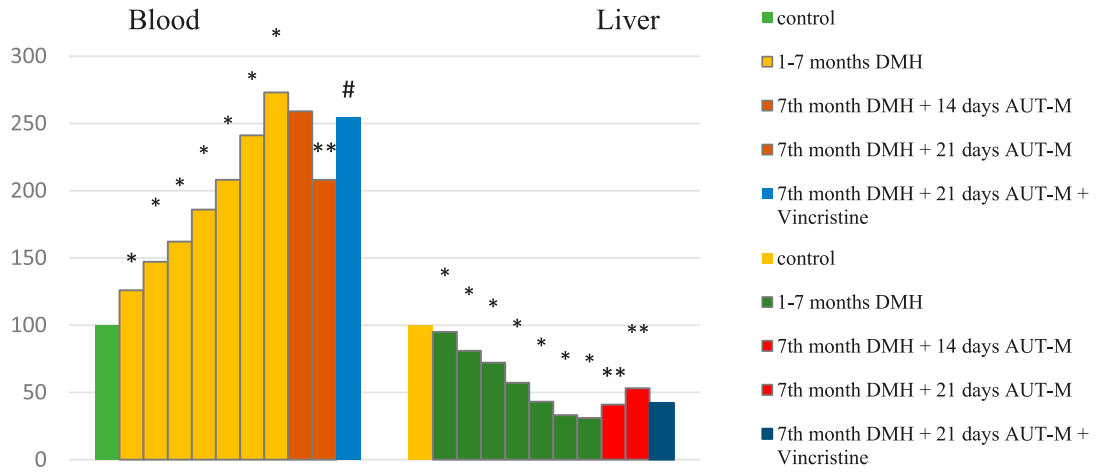

Figure 2. Dynamics of changes in the activity of AST in the serum and liver of rats with induced carcinogenesis after the use of cytostatics on the background of previous detoxification

In contrast, in the liver at all times of the experiment there is a probable progressive decrease in this indicator (3 months - by 42%, 5 months - by 59%, 7 months - by 66%) relative to the control group of animals ($p < 0.05$) Figure 1.

Similar changes were recorded in the study of the activity of the enzyme AST. After the 3rd month of modeling carcinogenesis, the activity of the studied indicator in the serum increased by 62%, at 5 months - by 107%, at 7 months - by 172% compared with the control animals Figure 2.

On the other hand, the activity of the AST enzyme in the liver homogenate significantly ($p < 0.05$) decreased by 28%, 57%, 69% compared with control animals in the corresponding terms of the study (Figure 2).

In animals that underwent a course of detoxification, there was a decrease in the manifestation of cytolytic syndrome. At 21 days of AUT-M administration in rats with carcinogenesis, there was a tendency to reduce the activity of AST in serum (by 65% relative to the level of affected animals) compared with the group of animals that were injected with carcinogen for 30 weeks. Restoration of enzyme activity in the liver of animals after 21-day extracorporeal detoxification (16% ALT, 22% AST) was also observed in comparison with the group of animals with DMH-induced cancer.

On the 14th day of cytostatic action in animals with simulated carcinogenesis on the background of enterosorption, minor changes in the activity of the studied enzymes in the direction of decreased activity in liver homogenate (ALT - by 10%) and increase in serum (ALT - by 17%, AST - by 18%).

During the simulation of carcinogenesis, a progressive increase in ALP activity in the serum

of DMH-affected rats was found compared to control animals.

Thus, the activity of ALP increased in the serum of DMH-affected animals: 1 month after carcinogen administration by 46%, 3 months - by 114%, 5 months - by 127%, 7 months - by 129% Figure 3. All changes were probable ($p < 0.05$).

In the liver homogenate, the activity of this enzyme probably decreased by 18% in 1 month of the experiment, by 5 months - by 50%, by 7 months - by 61% according to the control (Figure 3).

After application of the sorbent AUT-M for 21 days, the activity of ALP in the serum of affected rats probably decreased ($p < 0.05$) by 22%, in liver homogenate increased by 18% (Figure 3) compared with the group of animals from DMH-induced carcinogenesis.

The use of the cytostatic Vincristine on the background of 21-day correction with enterosorbent has little effect on changes in ALP activity. Thus, in the serum this figure increased by 21% compared with the group of DMH-affected rats after in vitro detoxification. In contrast, in the liver homogenate, ALP activity decreased slightly.

It was found that in the serum of animals with induced adenocarcinoma of the colon urea content probably ($p<0.05$) increased in all terms of the experiment (2 months - 1.1 times, 3 months - 1.2 times) relative to control indicators Table 1.

The maximum changes were observed after 4 months (increased 1.6 times) from the beginning of the modeling of the oncological process. In the subsequent terms of the experiment, this figure in the affected

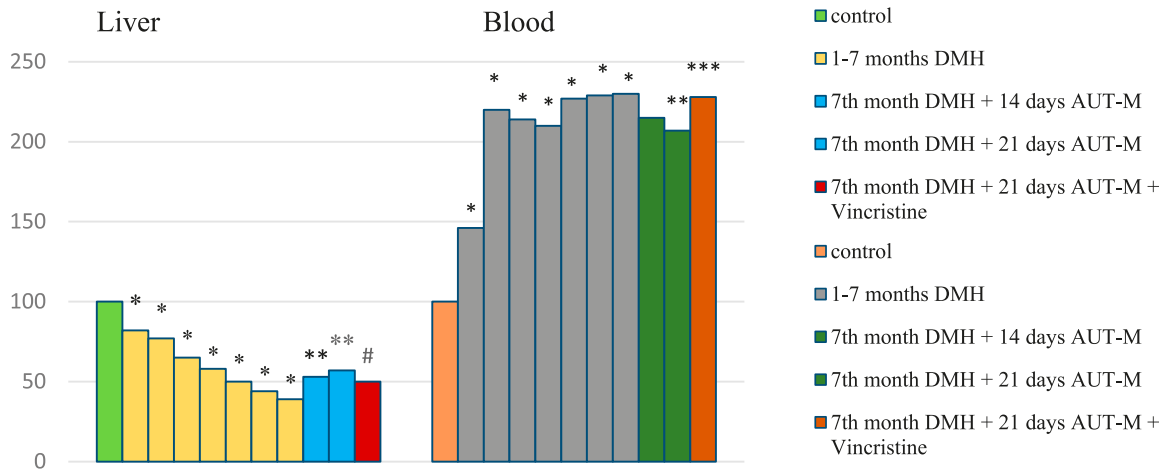

Figure 3. Dynamics of changes in the activity of ALP in the serum and liver of rats with induced carcinogenesis after the use of cytostatics on the background of previous detoxification

Table 1. The content of urea in the serum of rats with induced cancer and after the use of enterosorbent AUT-M and cytostatics Vincristine (M ± m)

Investigated indicator / Group of animals, term of affection	Urea, (mmol / l)
Control, n=7	40.96±1.11
1 Month, n=7	40.48±0.46
2 Month, n=7	47.00±0.85*
3 Month, n=7	49.08±0.53*
4 Month, n=7	51.68±0.76*
5 Month, n=7	46.13±0.78*
6 Month, n=7	46.09±0.84*
7 Month, n=7	45.91±0.62*
7 month DMH + AUT-M (14 days), n = 7	44.83±0.27
7 month DMH + AUT-M (21 days), n = 7	44.02±0.35
7 months DMH + AUT-M (21 days) + Vincristine (14 days), n = 7	46.35±0.55***

Note: * - probable changes between the indicators of animals of the control group and those affected by DMH; *** - probable changes between the rates of carcinogenic animals after enterosorption therapy (21 days) and animals receiving cytostatics (14 days).

animals decreased, but continued to exceed the level of the control group.

Extracorporeal detoxification with the drug AUT-M in the group of rats with induced chronic endotoxicosis is accompanied by normalization of urea content (without probable changes).

The use of the cytostatic Vincristine in animals with simulated carcinogenesis on the background of detoxification therapy contributes to a slight increase in urea content compared to a similar figure in the group of affected DMH animals on the background of 21-day detoxification.

DISCUSSION

It is known from the literature that the metabolism of the carcinogen DMH occurs in the liver. Accumulation of endogenous toxins formed during DMH metabolism becomes a trigger to change the permeability of hepatocyte plasma membranes, which is why the liver is most toxic in the early stages of carcinogenesis modeling [1, 18].

Aminotransferases are intracellular enzymes that are mainly deposited in the cytoplasm of hepatocytes, so their content in the serum is normally low [17]. According to our research, the simulation of DMH-induced carcinogenesis of the colon is accompanied by an increase in the activity of the studied enzymes in serum and a decrease in their activity in the liver in the early stages of cancer [4, 20, 21]

After 4 months of DMH administration, changes in aminotransferase activity in the studied tissues are less pronounced. Such data, in our opinion, may indicate a gradual decrease in the number of destroyed hepatocytes due to compensatory processes in the liver [17].

The hyperenzymemia we have identified indicates hepatocellular liver damage and the release of liver enzymes into the blood. An informative indicator of membrane destruction of hepatocytes is ALP. The rapid increase in the activity of the organ-specific enzyme ALP, which is localized mainly in the biliary tract, during the simulation of colon adenocarcinoma is, in our opinion, a consequence of inflammatory processes and liver cholestasis, as reported in the literature [15].

Elevated urea levels also indicate changes in liver function. Urea is the end product of protein metabolism, the content of which in the serum of experimental animals depends on the intensity of its synthesis and excretion [11]. After 30 weeks of DMH administration, the serum urea content of the affected animals increased by 12% relative to the control rat group. These data may indicate an increase in protein breakdown and the inability of the liver and kidneys to ensure inactivation and timely excretion of toxins.

Enterosorbent AUT-M reduces the load on the main organs of metabolism and detoxification (liver) and, accordingly, helps to activate the endogenous defenses of the body. The activity of the enzymes ALT, AST, ALP in the serum decreases, while in the liver - probably increases. Therefore, the sorption correction helps to stabilize the functional state of the liver.

It is believed that chemotherapy of malignant tumors is a drug-induced critical condition of the body, because all chemotherapeutics are poisons that are used to obtain cytoreductive, cytostatic or cytoeliminative effect. A number of authors note that hepatocytes are the most sensitive to chemotherapy. Under the action of cytostatics, the integrity of the plasma membranes of liver cells is violated, as a result of progressive cell-hepatic failure and cytolysis of hepatocytes [7, 11].

According to the results of our studies, the introduction of the cytostatic Vincristine on the background of 21-day use of enterosorbent did not lead to significant changes in the studied parameters. After 14 days of chemotherapy, the activity of ALT and AST in serum increased by 17% and 18%, respectively, in the liver, the activity of enzymes decreased compared to those in the group of animals with induced adenocarcinoma. Since the level of aminotransferase activity correlates with the degree of hepatocyte damage, the results obtained may indicate minor destructive changes under the influence of cytostatic correction against the background of the sorbent.

CONCLUSION

DMH-induced carcinogenesis of the colon in rats is accompanied by changes in the functional state of the liver, as indicated by an increase in urea in the serum of rats during seven months of modeling the cancer process. Increased activity of aminotransferases and alkaline phosphatase in serum and a decrease in these parameters in the liver of animals with a simulated cancer process indicates the development of cytolic syndrome and cholestasis.

In order to reduce the side effects of the cytostatic Vincristine, the enterosorbent AUT-M was used. The use of the sorbent has shown a significant potential for cytolysis inhibition in rats with DMH-induced colon carcinogenesis. Cytostatic correction after 21 days of extracorporeal detoxification indicates minor manifestations of cytolytic syndrome. The results confirm the positive effect of enterosorption in the development of carcinogenesis before the use of cytostatics and may serve as a basis for further study of the possibility of enteral sorption therapy in patients with colorectal cancer to reduce side effects of chemotherapy and alleviate the disease.

Conflicts of interest

The authors report no financial or any other conflicts of interest in this work.

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