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EXPERIMENTAL STUDY OF METALLOTHIONEIN SYNTHESIS IN MODELING HYPERTHERMIC INTRAOPERATIVE INTRAPERITONEAL CHEMOPERFUSION WITH CYSPLATINUM

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Abstract

In an experiment on white wild-type laboratory rats, it was shown that 90 minutes after intraperitoneal administration of cisplatin at a dose of 4 mg / kg as a solution heated to 43 °C, the integral level of metallothionein significantly increased in the organs. Metallothionein actively binds toxic compounds of metals, including platinum. This may be one of the main reasons for developing resistance to platinum drugs with repeated courses of chemotherapy. Fast inductive synthesis of MT with the introduction of heavy metal compounds is one of the mechanisms of adaptation of the organism to the introduction of a highly toxic platinum compound. The binding of platinum to the sulfhydryl groups of metallothionein reduces the side effect of cisplatin while simultaneously decreasing its antitumor activity. It is not recommended to use the main metallothionein inducers (Zn, Se, antioxidants) in patients with cisplatin sensitive tumors before chemotherapy courses.

Key words: cisplatin, kidneys, liver, metallothionein

According to the National Chancery of Ukraine (NKR) in the NKR Newsletter "Cancer in Ukraine" [1] at the beginning of 2018 there were 1219100 people registered for cancer, 129000 people were registered for the first time in 2017. In the Odessa region, 68,600

people, of which 7,100 of which the disease was detected for the first time in 2017. It is expected that in the next 20 years the number of new cases will increase by about 70 %. The number of cancer cases is projected to continue to grow from 14 million in 2012 to 22 million in the next decades.

The antitumor drug cisplatin has been widely used in the treatment of oncological diseases since 1978. Cisplatin is highly toxic as most heavy metal compounds. Due to faster metabolism in tumor cells and their active division, it is there that accumulates its highest concentration. Preparations of platinum have a pronounced damaging effect on the liver and especially the kidneys. It is dose-limiting for chemotherapy.

The possibility of reducing side effects with an increase in the antitumor effect of cisplatin led to the development and implementation of a method of intraoperative intraperitoneal chemo-perfusion (HIPEC). This is a new progressive method of chemotherapy, which consists in perfusion of the peritoneal cavity with solutions containing cytotoxic agents at a temperature above the physiological norm (41-43 ° C) [2, 3]. HIPEC is conducted 30-90 minutes. Cisplatin achieves its maximum effectiveness during this time. HIPEC is a method of adjuvant therapy and allows pharmacologically to destroy microscopic tumor lesions in the abdominal cavity, which inevitably remain after surgical removal of the tumor ("macroscopic cytoreduction"). With HIPEC, traditional drugs for chemotherapy are used (Cisplatinum, Doxorubicin, Mitomycin C, Irinotecan, Oxaliplatin). The effectiveness of their use in peritoneal carcinomatosis in this case is much higher than with intravenous administration [4] due to higher local concentrations near the tumor. Local hyperthermia, as a rule, causes a positive immune response, an increase in the absolute number of lymphocytes, an increase in the ratio of CD8 / CD4, restoring normal ratios of regulatory and effector lymphocytes, enhances the functional activity of T cells. The start of the cytokine cascade is confirmed by an increase in the plasma level of interleukins — 1, 2, 3, 4, 6, 7, 8, 10, 11, 12, interferons alpha, gamma, colony-stimulating factors (granulocyte and lymphocyte), tumor necrosis factors, etc. These peptides not only have independent antitumour activity, but also significantly enhance the effectiveness of some chemotherapy drugs — antimetabolites, anthracycline antibiotics, vinalkaloids. There are also data on induction of hyperthermia of apoptosis of tumor cells [5].

Unfortunately, with relapse of the tumor, there is often resistance to platinum drugs, although initially the tumor was sensitive. Resistance to cisplatin is explained by three molecular mechanisms: an increase in DNA repair, a change in cellular accumulation and increased inactivation of the drug due to inappropriate binding [6].

Sensitivity to platinum is returned if more than 2 years after the last chemotherapy with platinum (the probability that the patient will respond to platinum treatment, more than 70 %) [7].

The biochemical mechanism of the antitumor effect of cisplatin has been sufficiently studied. However, today there are no specific transmembrane vectors for platinum preparations and the ways of their cellular transport are unknown. Reduction of antitumor activity is due to the fact that cisplatin is excreted from the cell by means of the transmembrane transport system. In addition, cisplatin binds to sulfhydryl groups of proteins and peptides (for example, such as glutathione or metallothioneins) and ceases to damage the DNA of tumor cells, causing their apoptosis. Finally, cisplatin reacts nonspecifically with various subcellular components: proteins, RNA and DNA. Although the advantage of binding decreases in the RNA > DNA > protein range, a decrease in the concentration of cisplatin available for DNA binding leads to a decrease in antitumor activity.

Biomarkers of cisplatin resistance may be the cellular protein transporters of copper NER, CTR1 and CTR2, OCT2, ATP7A and ATP7B, glutathione GST and metallothioneins (MT) after additional clinical studies and standardization of methods and procedures of analysis [2].

The goal of our work — to determine the level of MT in liver and kidneys of rats in the modeling action of cisplatin with HIPEC.

Materials and methods. In experiments, rats weighing 220-250 g under anesthesia were intraperitoneally injected with a solution of cisplatin heated to 44 ° C in a dose of 4 mg / kg in physiological saline (1 mg / animal). The control group under similar conditions was given a 0.9 % sodium chloride solution in order to take into account the effects of anesthesia and other effects unrelated to platinum, which affect the induction of MT [8]. The animals of the experimental and control groups were withdrawn from the experiment after 90 minutes. The MT content in the liver and kidney homogenate was determined by the substitution method for measuring the concentration of cadmium after the complete displacement of zinc and copper from MT after the removal of high molecular weight proteins and low molecular weight compounds. The cadmium content was measured by the atomic-emission method [8].

Results and its discussion. The MT content in the liver and kidney of laboratory animals is given in Table 1. The body area for a person weighing 80 kg is approximately 1.93 ± 0.04 m2. Those. the usual dose of cisplatin with HIPEC 50-100 mg/m^2 is approximately 1.2-2.4 mg/kg. Given the lower specific sensitivity to cisplatin, the dose was increased to 4 mg / kg. In a preliminary experiment, it was found that this dose does not cause the death of

animals with a single intraperitoneal injection (LD50 = 6.2 mg/kg, death occurs after 8-10 days).

Table 1

The content of MT in the liver and kidneys after 90 min. after intraperitoneal administration of a solution of cisplatin at a dose of 4 mg/kg (*n* = 7, *P* > 0.95, T = 43 ° C)

	Liver		*kidney*	
Group	cisplatin	control	cisplatin	control
M (mean)	21,12	4,57	30,06	7,35
Std. off (σ)	2,96	0,71	2,47	1,08
Confidence interval	2,19	0,52	1,83	0,80
m	1,12	0,27	0,93	0,41
The maximum	25,70	5,55	33,11	9,12
The minimum	16,99	3,75	25,96	5,97

Note: the difference between experience and control is reliable, $p < 0,01$

The average content of MT in the liver and kidneys is 90 minutes after the intravenous administration of cisplatin increases, respectively, in 4.62 and 4.09 times. Previously, it was described that induction of MT synthesis can occur through several fundamentally different mechanisms. The process of expression of MT genes is activated through:

1. Metal response element (MRE)-binding transcription factor-1 (MTF-1), activated by "mobile" zinc ions. This transcription factor actively reacts to the entry of other toxic metals into the cell [9-11].

2. a glucocorticoid effector (GRE) [12],

3. Activating STAT proteins (signaling transducers and activators) through cytokine signaling;

4. antioxidants (or electrophiles), elements responsible for the reduction potential of cells (APE).

Physiological synthesis of MT occurs rapidly enough, increasing several times during cell proliferation. Probably, with the introduction of cisplatin, the first mechanism is realized primarily. Previously, it was shown that overexpression of the gene coding metallothionein occurs in 70.6 % of patients diagnosed with esophageal cancer. MT is associated with resistance to cis-platinum: in patients with MTN (+) tumors after treatment with cis-platinum,

the five-year survival was 26 %, and for MTH (-) -56 % [13, 14], i.e. Overexpression of MT significantly reduces the effectiveness of chemotherapy with cisplatin.

Conclusion. Fast inductive synthesis of MT with the introduction of heavy metal compounds is one of the mechanisms of adaptation of the organism to the introduction of a highly toxic platinum compound. The binding of platinum to the sulfhydryl groups of metallothionein reduces the side effect of cisplatin while simultaneously decreasing its antitumor activity. This means that patients with platinum-sensitive tumors are not recommended before the chemotherapy course to use the main inducers of metallothionein — preparations of zinc, selenium, and basic antioxidants.

Prospects for further research. It is necessary to study the kinetics of MT synthesis, to determine after what time after cisplatin administration the concentration of MT has decreased to the basal level.

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