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PATHOGENETIC SUBSTANTIATION OF THE ANTI-TUMOR EFFECT **OF DIHYDROQUERCETIN**

Under experimental conditions on malignant cultures malignant cells has been demonstrated in experimental hydroquercetin malignant tumoral cell cultures. peroxidation in malignant cells.

The antitumoral activity of a natural bioflavonoid in associated with the activation of lipo- conditions at the

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An urgent problem of modern antitumor treatment of oncological patients is the search, study of the mechanisms of action and development of regimens for the use of drugs with antioxidant and immunomodulatory properties based on non-toxic

natural substances,
such as bioflavonoids [1]. This group includes dihydroquercetin, a lipophilic substance with high antioxidant and P-vitamin activity [2, 3]. In terms of the severity of the antioxidant effect, the drug is equal to ÿ-tocopherol and is more active than ÿ-carotene. With various pathological conditions

standing dihydroquercetin may exhibit
itself as an effective means of maintenance therapy, is able to regulate the reactions of the immune system, provide antiviral

noe, anti-allergic and anti-inflammatory
positive effect, reduce the degree of endotoxemia [4]. To include it as an additional component of antitumor therapy in the complex and combined

treatment of patients suffering from malignant
neoplasms, it seems appropriate to study the antiproliferative effect of the drug on cells of various types of blastomas.

The aim of the study is to evaluate the anti-opioid
cholic activity of dihydroquercetin on culture
tours of malignant tumor cells.

Materials and methods. As an experiment
mental cell lines were used
cell cultures HEP-2 (epidermal carcinoma of the larynx) and Hela (carcinoma of the cervix), obtained from the Nizhny Novgorod enterprise for the production of bacterial preparations ImBio, a branch of the Federal State Unitary Enterprise NPO Microgen of the Ministry of Health of the Russian Federation, which has a license for the production of cell culture (production certificate No. 001420.02-73024). When growing the cells, medium 199 was used. For both cell lines, the inoculum dose was 50–100 cells per 1 ml, the sieving ratio was 1:4–1:10. The cells were harvested using Versen's solution (4/5) with 0.25% trypsin; the passage frequency was 5-7 days. Cell transfers were performed under sterile conditions. Counting the number

cells were carried out in suspension using
using the Goryaev camera at a magnification of 150x250. Cell viability was judged by their staining with 0.1% trypan blue solution in saline. At first

On the day of the experiment, the cells were transplanted onto slides placed in test tubes with a nutrient medium and incubated at a temperature of 37°C for 48 h.

On the third day of the experiment, dihydroquercetin was weighed 150; fifty; twenty; 10; 5 and 2.5 mg were dissolved in 10 ml of a solution (5 ml of 0.9% NaCl + 5 ml of nutrient medium), placed in sterile flasks, and dissolved in a water bath for

5 min. From test tubes with glasses on which
cells were found, the old nutrient was removed
Wednesday. To each tube was added 2 ml of
brine solution containing dihydroquerce
tin, so that the glasses are completely immersed in the liquid. Cell lines placed in 2 ml of nutrient medium (series 1) and cell lines placed in 2 ml of a mixture consisting of physiological saline (0.9% NaCl) and nutrient medium in the ratio 1:1 (2nd series). Control tubes

and experimental media were closed with tightly pressed rubber stoppers (to maintain sterility) and placed in a thermostat for 48 h at a temperature of 37°ÿ.

On the fifth day of the experiment, test tubes from
taken out of the thermostat, the glasses were taken out and taken out
dried in the open air. Fixative preparations
stained with a solution of eosin-methylene blue
according to May-Grunwald and stained with azure-eosin dye according to Romanovsky for 3 min. Next, the glasses were washed with cold running water, dried in air, and examined.

fall into the microscope.

The intensity of free-radical reactions
ions were determined by the method of induced biochemiluminescence on a BKhL-06 instrument. At
In this case, we analyzed the parameters characterizing
lipid peroxidation levels
(LPO): Imax is the maximum luminescence intensity, which shows the potential ability of a biological object to peroxide lipids, S is the light sum, reflecting

content of radicals in the plasma corresponding to
terminating the free-radical oxidation chain is inversely proportional to the antioxidant activity of the sample; tg-2a - indicator, character

measuring the decay rate of free radical oxidation processes in plasma; Imax/S. Kro¬
In addition, we measured the levels of molecular LPO products in the cell culture homogenate: diene conjugates (DC), triene conjugates (TC), and Schiff bases (SS). To do this, the liquid was drained from the test tubes of each series, and 0.5 ml of a physiological solution was added to the remaining cells, under the action of which

cells were destroyed.

Results and discussion. Microscopic examination data showed that 77% of viable cells were preserved in series 1, and 23% of the total number of dead cells. In the 2nd series, these indicators were 79 and 21% of the

responsibly.

In the case of using weighed portions of dihydroquercetin in 50 and 150 mg, intense staining of preparations was observed, apparently

sti associated with an increase in the permeability of cell membranes. There were no degenerative changes in the cells, the peculiarity was

pronounced adhesion of cells to the surface
glass (the phenomenon of "welding"). Visible were the crystals of the drug that formed the precipitate. In this connection, in what follows, we took into account the results
The results of the experiment with smaller sample weights
paratha.

The data of microscopic examination of these cell lines showed that the antitumor effect of dihydroquercetin began to manifest itself at the lowest concentration of the drug, 2.5 mg. At the same time, the cells reveal

smeared nuclei and the onset of karyolysis appeared, unchanged cells remained only in 7% of cases (Table 1).

With an increase in the sample of the drug up to 20 mg

Table 1

Microscopic changes in tumor cells exposed to different concentrations of dihydroquercetin, %

Microscopic signs	Concentration of dihydroquercetin, mg			
	2.5	five	10	twenty
Naked kernels	five	twenty	17	eleven
Cells without a nucleus, cytoplasm and cell membrane preserved	21	23	five	15
The contours of the nucleus are blurred, karyolysis has begun	32	38.5	15	53
Hyperchromic staining, cell structures are not distinguishable	2	—	7.5	15
The contours of the nucleus are broken or it is fragmented, the cytoplasm is fragmented, the cell membrane is destroyed	33	fourteen	50.5	eleven
Unaltered cells	7	five	10	2

Table 2

Indicators of lipid peroxidation and antioxidant defense system in tumor cells of control and experimental series

Series	Indicators						
	tg-2a	1max, mV/s	S, mV/30 s	Imax/S	DC	TC	OSH
Control 1	0.60±0.007	0.23±0.013	2.92±0.12	0.29±0.11	0.07±0.001	0.019±0.0010	6.9±0.98
Control 2	0.83±0.004	0.26±0.015	2.74±0.19	0.23±0.10	0.10±0.002	0.02±0.0012	4.0±0.75
Experiment 1.5 mg	0.50±0.003+	0.90±0.006*+	3.95±0.21*+	0.25±0.13	0.10±0.003	0.03±0.0013	2.5±0.63*+
Experiment 2, 10 mg	0.71±0.004+	1.49±0.007*+	4.91±0.32*+	0.41±0.11*+	0.12±0.002	0.05±0.0012*+	2.62±0.54*
Experiment 3, 20 mg	0.67±0.10+	1.46±0.006*+	4.34±0.51*+	0.34±0.09+	0.12±0.003	0.05±0.0011*+	2.62±0.81*

* — differences are significant with the control series 1 (ÿ<0.05); + — differences are significant with the control series 2 (ÿ<0.05).

the number of unchanged cells decreased to 2%, and the number of cells with a blurred contour and the onset of karyolysis was 53%.

The presented data indicated that the addition to the nutrient medium, approx. destroying tumor cells, dihydroquerce mud contributed to the development of destructive changes in them, which eventually led to death, i.e. the drug had an antitumor effect.

To clarify the mechanism of the antitumor action of dihydroquercetin, the effect of the drug on the processes of free-radical oxidation was evaluated. An analysis of the results of the studies performed showed that the use of dihydroquercetin solutions favors a significant activation of lipid peroxidation reactions in tumor culture cells compared to with the corresponding indicators obtained mi in tumor cells of the control series (Table 2). A dose-dependent effect of the drug was clearly observed. For example, after weighing 5 mg of dihydroquercetin, the value of Imax, which characterizes the activity of free-radical reactions, increased by more than 3 times (p < 0.05); when using weighed portions of 10 and 20 mg - 5 times (p<0.05) compared with the value of this parameter in the experimental groups. Simultaneously, the level of primary LPO products — DC increased, which indicated the activation of the initial processes of lipid peroxidation. An increase in S and Imax/S indicated a decrease in the activity of the antioxidant defense system in tumor cells. The latter was the reason activation of lipid peroxidation processes and, possibly, contributed to the launch of apoptotic reactions, which ultimately led to the destruction and cell death.

Conclusion. Dihydroquercetin prodemon strated antitumor activity on cultures of malignant tumor cells, associated with activation of lipoperoc processes sidation in malignant cells.

LITERATURE A

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