Effects of T-Activin and Vitamin E on Toxicity and Antitumor Activity of Cyclophosphamide

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We studied the effect of combination treatment with T-activin and vitamin E on acute toxicity and antitumor activity of cyclophosphamide in mice. Combined administration of these preparations 1.37-fold increased the maximum permissible dose of cyclophosphamide without affecting its LD_{50} and delayed mouse death from cyclophosphamide toxicity. Most mice died only 3 days after combination treatment with the test preparations and cyclophosphamide in doses of LD_{16}-LD_{84}. The second peak of death from hematologic toxicity of cyclophosphamide was absent under these conditions. T-activin and vitamin E did not abolish the antitumor effect of cyclophosphamide on mice with subcutaneously implanted P-388 lympholeukemia. Tumor growth was suppressed by 100%.

Key Words: *cyclophosphamide; vitamin E; T-activin; toxicity-modifying effect; antitumor activity*

The opportunity for successful intensive-dosage and high-dosage chemotherapy of patients with tumors is diminished due to high toxicity of antitumor drugs [3,4,6,7]. Prevention of side effects of cytostatics without diminishing their antitumor activity will improve the quality of life in patients. Immunosuppressive effect on bone marrow hemopoiesis and development of neutropenia and cytopenia are the major complications of chemotherapy [3]. Hepatotoxicity of cytostatics also attenuates the antitumor effect and increases toxicity of preparations metabolized in the liver [4]. Preparations with hepatoprotective and immunomodulatory properties (*e.g.*, vitamin E and T-activin) are used to reduce the incidence and severity of complications after cytostatic therapy [1,2,5,8,9]. Cytostatic-induced immunosuppression and myelosuppression in mice and rats is modeled by administration of cyclophosphamide in high doses.

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Here we studied the effect of combination treatment with vitamin E and T-activin on cytotoxicity and antitumor activity of cyclophosphamide in mice.

MATERIALS AND METHODS

Experiments were performed on 170 (CBA×C57BL/6J) F_1 mice weighing 18-22 g and obtained from the Svetlye Gory nursery. The animals were kept in a vivarium (G. F. Gauze Institute of Search for New Antibiotics) and had free access to water and standard briquetted extruded feed.

We used pharmaceutical preparations vitamin E and T-activin. Vitamin E was administered intragastrically 3 times in a single dose of 5 U at 48-h intervals. T-activin was dissolved in physiological saline (NaCl) and injected intramuscularly in a single dose of 0.25 mg/kg for 5 days. Cyclophosphamide was injected intraperitoneally in single doses of 200-700 mg/kg.

The test preparations were administered in combination with cyclophosphamide to evaluate their toxicity-modifying effect. We studied whether vitamin E and T-activin can modify the toxic dose of cyclophosphamide. Toxic doses LD_{50} and LD_{10} (minimally per-

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missible doses, MPD) were estimated by the method of Litchfield and Wilcoxon with modifications of Z. Rott using nomograms. Each group consisted of 12 animals. Observations were performed for 1 month. The number of mice dying from cyclophosphamide in doses of LD_{16}-LD_{84} was taken as 100%. We constructed the curve for the number of animals dying in various periods of observations. The periods of peak mortality were evaluated. The degree of toxicity was estimated by the survival curve (probit model).

The effect of combination treatment with vitamin E and T-activin on antitumor activity of cyclophosphamide was studied in mice with subcutaneously implanted P-388 lympholeukemia. The cells were obtained from the Collection of Tumor Strains (N. N. Blokhin Russian Oncological Research Center). The mice were implanted with 1 billion tumor cells. Cyclophosphamide in a single dose of 200 mg/kg was injected 48 h after tumor implantation. The antitumor effect was evaluated by tumor growth inhibition (TGI) and increase in the mean lifespan (LS). These indexes were compared in cyclophosphamide-treated mice and animals not receiving specific therapy and expressed in percents. The results were analyzed by Fischer-Student test with modifications of R. B. Strelkov. The differences were significant at $p<0.05$.

RESULTS

LD_{50} and MPD (LD_{10}) of cyclophosphamide were 387 [345.5÷433.4] mg/kg and 236[210.7÷264.3] mg/kg, respectively. The peak mortality rates were observed on days 3 and 5. The preparation exhibited intestinal and hematologic toxicity. Animal death was described by a biphasic curve, which is typical of cyclophosphamide-induced changes (Fig. 1).

Combined administration of T-activin and vitamin E had no effect on LD_{50} of cyclophosphamide (397 [342.2÷460.5] mg/kg), but increased its MPD (LD10) by 1.37 times (324[279.3÷375.8] mg/kg).

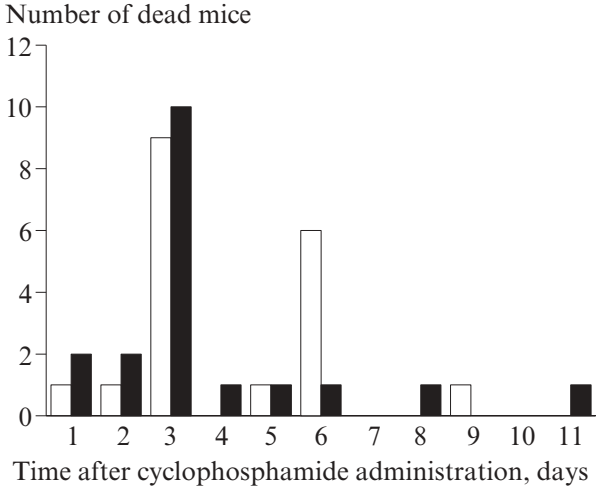

Fig. 1. Number of dead mice and time of death after treatment with cyclophosphamide alone (light bars) or in combination with vitamin E and T-activin (dark bars).

The survival curve showed that T-activin and vitamin E widened the toxic effect of cyclophosphamide due to increase in low toxic doses. It should be emphasized that most mice died only 3 days after combination treatment with the test preparations and cyclophosphamide in doses of LD_{16}-LD_{84}. The second peak of death was absent under these conditions.

Vitamin E and T-activin practically did not change the efficiency of cytostatic therapy (Table 1). The primary tumor response and survival of mice with subcutaneously implanted P-388 leukemia did not differ after therapy with cyclophosphamide alone or in combination with vitamin E and T-activin. TGI and LS reached 99-100 and 259-264%, respectively.

Our results show that combined administration of T-activin and vitamin E increases toxic activity of cyclophosphamide, but has no effect on the efficiency

TABLE 1. Therapeutic Efficiency of Cyclophosphamide Administered in Combination with T-activin and Vitamin E to Mice with P-388 Lympholeukemia (M±m)

Group, number of mice	Average tumor size after therapy, mm^3			LS, days	LS, %
	day 5	day 10	day 14		
Control (n=8)	247 [194÷300]	1616 [743÷2489]	2867 [2140÷3594]	17.1 [16.5÷17.1]	100
Cyclophosphamide, 200 mg/kg (n=8)	2 [0÷4]	9 [3÷15]	13 [4÷22]	44.6 [41.0÷48.2]	259*
T-activin (0.25 mg/kg) and vitamin E (5.0 U, n=8)	145 [149÷241]	1080 [306÷1854]	670	16.6 [16.0÷17.2]	97
Cyclophosphamide (200.00 mg/kg), T-activin (0.25 mg/kg), and vitamin E (5.00 U, n=10)	Without tumor	Without tumor	Without tumor	45.3 [42.8÷47.8]	264*

Note. *$p<0.05$ compared to the control.

of cytostatic therapy. Combination treatment with T-activin and vitamin E modifies hematologic toxicity of cyclophosphamide and prevents suppression of hemopoiesis and death of animals from secondary infection. T-activin and vitamin E delay the toxic effect of cyclophosphamide. The influence of combination treatment with T-activin and vitamin E requires further detailed investigations.

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