Effects of Cisplatin on Lymphocyte Structure and Functions in Mice with Ehrlich Ascitic Carcinoma

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The effects of cisplatin on $[Ca^{2+}]_{in}$, pH_{in}, NAD(P)H level, and lymphocyte membrane microviscosity were studied in mice with Ehrlich ascitic carcinoma. The level of free $[Ca^{2+}]_{in}$ in lymphocytes from mice with Ehrlich ascitic carcinoma was 4-fold reduced compared to that in intact animals on day 13 of tumor development, while $[H^+]_{in}$ level was elevated. Cisplatin caused no changes in the level of free Ca^{2+}, but reduced cytosol acidification. Lymphocyte membrane fluidity in mice with tumors was increased in the lipid bilayer and in the protein-lipid contact zone and did not depend on cisplatin treatment. The level of NAD(P)H was low in mice with tumors, but increased sharply after cisplatin treatment. It seems that functional activity of lymphocytes decreased at the stage of well-developed tumor, which promoted inhibition of the lymphocyte defense properties. Cisplatin did not modify the structure and functions of lymphocytes and presumably even improved their energy status.

Key Words: *lymphocytes; calcium cations; cisplatin; Ehrlich ascitic carcinoma*

Cisplatin is an antitumor drug, a platinum(II) compound widely used in practical oncology for tumor growth inhibition [5]. However, cisplatin treatment is sometimes associated with certain side effects, for example, renal dysfunction, anaphylactic reactions, leukopenia, thrombopenia, anemia, and neuropathies, as it is not a compound specific towards tumor cells and diffuses into all biological fluids and tissues of the body [8]. The toxic effect of cisplatin on organ and tissue functions is primarily determined by its antiproliferative effect on all rapidly dividing cells, including lymphocytes. Therefore, evaluation of the effects of this widely used antitumor drug on lymphocyte status is an important problem.

We studied the effects of cisplatin on lymphocyte structure and functions in mice with Ehrlich ascitic carcinoma.

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MATERIALS AND METHODS

The following reagents were used in the study: HEPES, Fura-2AM, digitonin, EGTA, pyrene, fluorescein isothiocyanate (FITC), Ficoll (Sigma); cisplatin (Ebewe); NaCl, KCl, $CaCl_2$, $NaHCO_3$, Na_2HPO_4, KH_2PO_4, $MgSO_4$, glucose analytically pure grade (Reakhim).

Experiments were carried out on ICR male albino mice (27-35 g) from Vector Center Breeding Department. Animals of groups 1 and 2 (14 per group) were intraperitoneally transplanted 3×10^6 ascitic cells. Intact animals (n=14; group 3) served as the control. All animals were injected with 5% glucose solution throughout the entire experiment. Group 2 mice received, in addition to glucose, 500 μg cisplatin in saline on days 3, 5, 7, 9, and 11 of tumor development. Lymphocytes, isolated from mice on day 13 of tumor development, were the object of the study. The mice were narcotized with ether, after which blood was collected from the carotid artery. Lymphocytes were isolated from the

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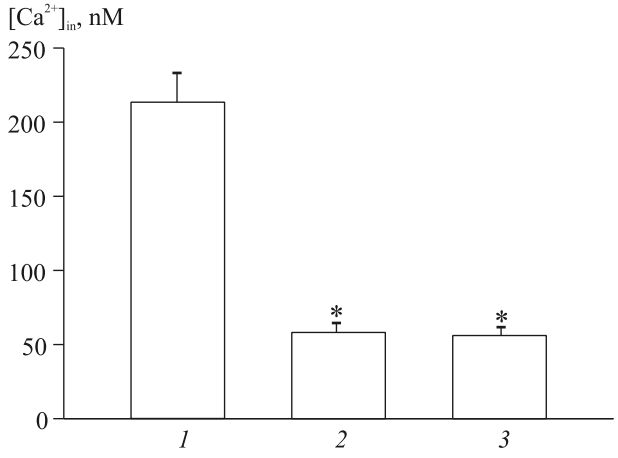

Fig. 1. Lymphocyte $[Ca^{2+}]_{in}$ content in intact mice (*1*), mice with Ehrlich ascitic carcinoma (*2*), and mice with Ehrlich ascitic carcinoma treated with cisplatin (*3*). **p*<0.001 compared to control.

blood on Ficoll gradient, after which the cells were counted by the standard method in a Goryaev chamber.

Intracellular calcium concentration was measured using Fura-2AM fluorescent probe on an Aminco Bowman Series 2 spectrofluorometer (Thermo Spectronic) at 25°C [3].

The level of pH was evaluated using FITC [7] as follows. Lymphocytes were incubated in Hanks' solution with 1 mg/ml FITC: 15 min at 4°C, 10 min at 37°C. Extracellular stain was removed by 3-fold washout with cold buffer. Before measurements, the cells were kept on ice. Stained washed lymphocytes (10^6 cells) were resuspended in the same buffer and the measurements were carried out directly on an Aminco Bowman Series 2 spectrofluorometer (Thermo Spectronic) at λ_{ex}=493 nm and λ_{abs}=520 nm.

Microviscosity of the lymphocyte membrane lipid bilayer and protein-lipid contact zone was evaluated using pyrene fluorescent probe. Pyrene eximerization coefficient J_e/J_m equal to the ratio of eximer J_e (λ_{em}=470 nm) to monomer J_m (λ_{em}=395 nm) fluorescence intensities was in inverse relationship with the microviscosity. The cell membrane lipid bilayer microviscosity was evaluated at λ_{ex}=334 nm, protein-lipid contact zone microviscosity at λ_{ex}=282 nm [1] on an Aminco Bowman Series 2 spectrofluorometer (Thermo Spectronic).

The level of NAD(P)H was measured by its own fluorescence. The measurements were carried out an Aminco Bowman Series 2 spectrofluorometer (Thermo Spectronic). Each sample contained 200,000 washed lymphocytes resuspended in 2 ml Hanks' solution. The fluorescence of NAD(P)H was recorded at λ_{ex}=365 nm and λ_{em}=450 nm [9].

The results were statistically processed using Origin 5.0 and Statistica 7.0 software. The significance of differences was evaluated by the nonparametric Mann–Whitney test.

RESULTS

A certain spectrum of physicochemical parameters (membrane potential, pH, membrane viscosity, redox status, intracellular Ca cation concentration, *etc.*) corresponds to each functional state of the cell. The first event in response to mitogen stimulation of membrane receptors is elevation of Ca^{2+} level in the cell [10], the level of free $[Ca^{2+}]_{in}$ increasing directly after lymphocyte stimulation and remaining high during several hours. Ca^{2+} act by regulating activities of phospholipases, proteases, endonucleases, *etc.*, thus coordination various signal pathways for the formation of adequate cell response [6]. Hence, $[Ca^{2+}]_{in}$ is a key factor of modulation of the cell functional status [4].

Experiments showed that free $[Ca^{2+}]_{in}$ in the lymphocyte cytosol of mice with tumors on day 13 of Ehrlich carcinoma development was 4-fold reduced compared to the control (Fig. 1), which attested to inhibition of the lymphocyte functional activity and host inability to control tumor growth. Cisplatin did not change lymphocyte $[Ca^{2+}]_{in}$ (Fig. 1).

Ca^{2+} concentration in cells directly depends on physicochemical characteristics of the cell membranes, because they determine the work of membrane-bound

TABLE 1. Effect of Cisplatin on Lymphocyte Membrane Microviscosity (J_e/J_m) in the Lipid Bilayer and Protein-Lipid Contact Zones

Group of animals	Microviscosity, J_e/J_m, rel. units	
	lipid bilayer	protein-lipid zone
Control (*n*=13)	0.24±0.02	1.70±0.10
Ehrlich ascitic carcinoma (*n*=10)	0.42±0.01*	2.43±0.19*
Ehrlich ascitic carcinoma+cisplatin (*n*=10)	0.40±0.02*	2.41±0.09*

Note. **p*<0.001 compared to control.

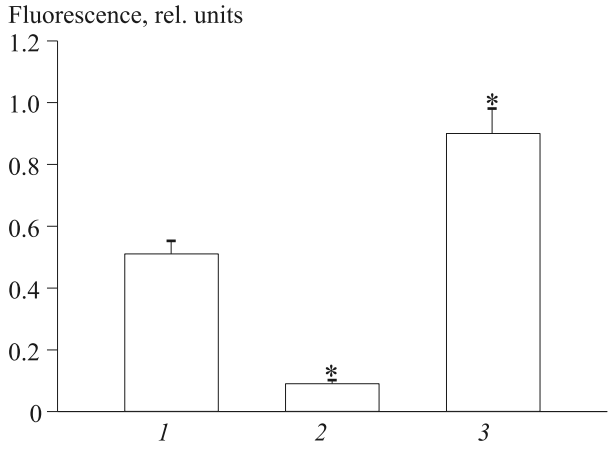

Fig 2. NAD(P)H content in lymphocytes of intact mice (*1*), mice with Ehrlich ascitic carcinoma (*2*), and mice with Ehrlich ascitic carcinoma treated with cisplatin (*3*). *p<0.001 compared to control.

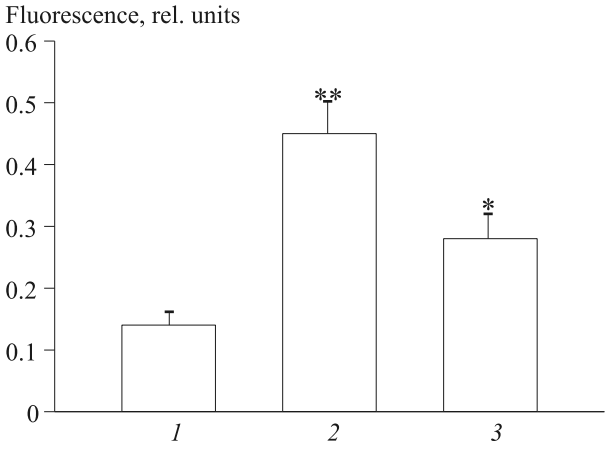

Fig. 3. $[H^+]_{in}$ in lymphocytes of intact mice (*1*), mice with Ehrlich ascitic carcinoma (*2*), and mice with Ehrlich ascitic carcinoma treated with cisplatin (*3*). *p<0.005, **p<0.001 compared to control.

enzymes, channels, and receptors. Lymphocytes modulate the physicochemical characteristics of membranes many times throughout the vital cycle; for example, the lymphocyte membrane becomes more fluid during their stimulation, due to which the lymphocytes more effectively realize their functions. That is why evaluation of the membrane viscosity is essential for evaluation of the lymphocyte function. Our results indicated that lymphocyte membranes on day 13 of tumor development were more fluid than in the control, because pyrene eximerization coefficient in the protein-lipid contact zone was 40% higher (p<0.05) and in the lipid bilayer zone 43% higher (p<0.05) than in control cells. Cisplatin did not change the lymphocyte membrane viscosity (Table 1).

The level of NAD(P)H indirectly characterizing the oxidized to reduced substrate ratio reflects lymphocyte functional status, because the cell redox status determines activities of numerous intracellular proteins [2] and hence, the cell status. In our study, the level of NAD(P)H in lymphocytes from mice with Ehrlich ascitic carcinoma was significantly lower than in intact animals (Fig. 2), which indicated modification of the lymphocyte redox status under conditions of tumor growth. Presumably, it was caused by lower content of reduced substrates in the cell because of intense absorption of glucose, amino acids, cholesterol, and other substrates by tumor cells and low levels of these substances in the plasma. Hence, the concentrations of reduced substrates in the lymphocytes were low. The level of NAD(P)H increased sharply in the lymphocytes of mice treated with cisplatin and was even higher than in the control. Two explanations of this phenomenon are possible. First, cisplatin inhibited tumor growth and less intensive consumption of energy substrates by the tumor could lead to elevation of glucose level in the plasma and hence, in lymphocytes, which inevitably promoted the increase in the levels of reduced substrates, including pyridine nucleotides. Second, cisplatin is an effective inhibitor of oxidative processes in lymphocytes, which also presumably led to an increase of NAD(P)H level.

In order to evaluate more precisely the lymphocyte function, we evaluated one more parameter characterizing cell activity, $[H^+]_{in}$. It is well known that pH_{in} elevation is an early universal mechanism responsible for mitogenic stimulation [11], while acidification of the cytoplasm characterizes apoptosis [12]. In our study the level of lymphocyte $[H^+]_{in}$ in mice with Ehrlich ascitic carcinoma was significantly higher than in intact animals (Fig. 3). It remained higher than in the control under conditions of cisplatin treatment, despite reduction of the lymphocyte cytosol oxidation. Hence, the results suggested that metabolic changes in the lymphocyte population of mice with carcinomas at the stage of well-developed tumor could promote their apoptotic death. However, decreased intracellular levels of Ca cations in lymphocytes observed in our experiments contradicts this hypothesis, though these cations (as is well known) activate the enzymes involved in triggering of apoptosis cascade reactions.

Hence, our data indicate that functional activity of lymphocytes is reduced in the organism with well-developed Ehrlich carcinoma. Cisplatin treatment did not deteriorate lymphocyte function and presumably even improved their energy status.

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