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Decrease in Bcl-2 Protein Level during the Development of Lewis Carcinosarcome

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We studied the development of Lewis carcinoma and possible antitumor effect of preliminary administered antioxidant anphen. The tumor was intramuscularly transplanted to C57Bl×DBA mice (7×10^6 cells per mouse). According to immunoblotting results, the content of anti-apoptotic Bcl-2 protein steadily decreased starting from post-transplantation day 11. In few days, its content decreased by 15-20% and soon the animals died. After administration of anphen, the content of Bcl-2 decreased more rapidly than in the control. Atomic force microscopy revealed a decrease in the mean volume of erythrocytes and then increase in this parameter at the terminal stage of tumor growth. These findings suggest that anphen does not affect the tumor growth rate and mouse lifespan, but enhances apoptosis of blood cells of animals with Lewis carcinoma at the terminal stages of tumor growth.

Key Words: *Lewis carcinosarcoma; Bcl-2 protein; anphen*

Previous studies showed that some synthetic antioxidants from the class of sterically hindered phenols synthesized at the N. M. Emanuel Institute of Biochemical Physics, exhibit antitumor activity. The positive effect of 4-fold administration of antioxidant phenosan in spontaneous leukemia of AKR mice was reported [4,5]. The changes in the parameters of the tumor process were evaluated by the serum content of main apoptosis regulators p53 and Bcl-2. Administration of phenosan was followed by an increase in the level of apoptotic protein Bcl-2 and a decrease in the content of double-stranded DNA breaks [3,4]. At the same time, antioxidant anphen (sodium 1-(carboxy)-1-(N-methylamide)-2-(3',5'-di-tret-butyl-4'-hydroxyphenyl)-propanate) administered to mice after sarcoma-37 transplantation led to complete suppression of tumor growth [2].

It is known that Bcl-2 protein inhibits apoptosis in cells, modulates redox processes, and, in the presence of ROS excess in the blood, acts as an antioxidant and anti-inflammatory protein. There are ample data that tumor growth and aging are associated with activation of apoptosis over repair processes and that Bcl-2 content in animals decreases with age [7-9]. Expression of p53 protein and a decrease in Bcl-2 level were detected in tissues of different tumors, including tumors of the upper respiratory tract and lungs, and in many cell lines, *e.g.* human lung carcinoma TW2.6 [7,8]. A prognostic value of p53 and Bcl-2 induction in operable cancer of lung was reported [9].

At the same time, some repair processes are associated with increased content of anti-apoptotic protein Bcl-2. This is consistent with the idea on anti-inflammatory and antioxidant function of Bcl-2 family proteins in the blood [6]. In cells with enhanced expression of Bcl-2, the concentrations of hydroxyl radicals decrease, the level of glutathione increases, and LPO is suppressed [6].

Here we studied the effect of Lewis carcinoma growth on plasma content of anti-apoptotic protein Bcl-2 and erythrocyte morphology in animals pretreated with anphen [2] in comparison with untreated controls.

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MATERIALS AND METHODS

The study was performed on 3-4-months-old C57Bl× DBA hybrid mice weighting 25 g. The animals received transplantation of Lewis lung carcinoma cells in a dose of 7×10^6 per mouse. Anphen (1 mg/kg) was injected into the thigh over 4 days before the transplantation of the tumor cells.

The development of the tumor process was assessed by changes in volume of the tumor growing in the thigh. The volume of the subcutaneous tumor was determined from three diameters, assuming that the tumor is an ellipsoid.

The anti-apoptotic protein Bcl-2 in the blood plasma was assayed by immunoblotting. Electrophoresis of plasma proteins was conducted in 10% SDS gel in the Laemmli system. Monoclonal Anti-BCL-2 clone 10C4 and horseradish peroxidase-labeled anti-rabbit IgG (Sigma) were used as the primary and secondary antibody, respectively. AES Staining Kit (Sigma-Aldrich) was used for protein detection on nitrocellulose membrane. The optical density of protein bands was determined using BMP scale software.

Changes in erythrocyte morphology were evaluated by atomic force microscopy (AFM). Erythrocytes were fixed with 2% glutaraldehyde for 30 min and air-dry preparations of erythrocytes on silicon substrate were studied [1]. The measurements were conducted on a SOLVER P47 SMENA instrument, at 150 kHz in a semi-contact mode with a NSG 11 cantilever.

The results were processed using ImageAnalyzis and Statistica 6.0 software.

RESULTS

The level of Bcl-2 protein remained stable within a few days after transplantation, the appearance of the tumor was recorded from day 7. On day 11, a significant decrease in the content of anti-apoptotic protein Bcl-2 was revealed; on day 18, its level decreased by 15-20%, and the animals died soon. This is consistent with the data on a decrease in Bcl-2 level in lymphocytes during aging and during tumor process [7-9]. Thus, we observed an expected decrease in the level of Bcl-2 protein during the development of transplanted solid Lewis carcinoma at the final stage of its development.

After pretreatment with anphen, the content of Bcl-2 protein also decreased starting from day 11, but this decrease was abrupt than in the control (Fig. 1). These changes in protein level were probably related to the observed process of apoptosis in the last stages of tumor growth.

AFM also revealed changes in erythrocyte morphology during tumor development. Erythrocyte volume did not decrease on days 1-8 (Fig. 2), on day 11 (at the terminal stage of tumor growth), the mean erythrocyte volume decreased in the control and anphen-pretreated animals; immediately before animal death, the erythrocyte volume increased and echinocytes, stomatocytes, and other pathological forms of erythrocytes appeared.

Kinetic curves of Lewis carcinoma growth (Fig. 3) in the control and after anphen administration also practically coincided. These data may indicate that a preliminary intramuscular injection of 1 mg/kg anphen did not affect the rate of tumor growth and mouse lifespan (data not shown).

The effect of anphen in this experiment differed from that of phenosan [4] after similar preliminary administration and from the effect of anphen in animals with sarcoma-37 [2]. Thus, antioxidants derivatives of the same class can exhibit different antitumor activity

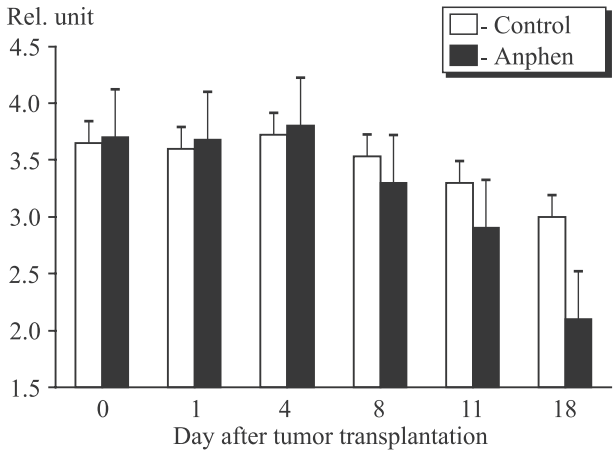

Fig. 1. Change in Bcl-2 protein content in mouse plasma during Lewis carcinoma growth in the control and after administration of anphen (1 mg/kg).

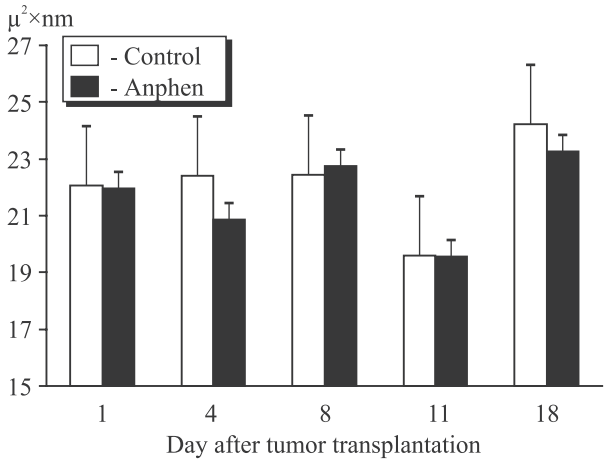

Fig. 2. Changes in erythrocyte volume assessed by AFM during the development of Lewis carcinoma in the control and after administration of 1 mg/mg anphen.

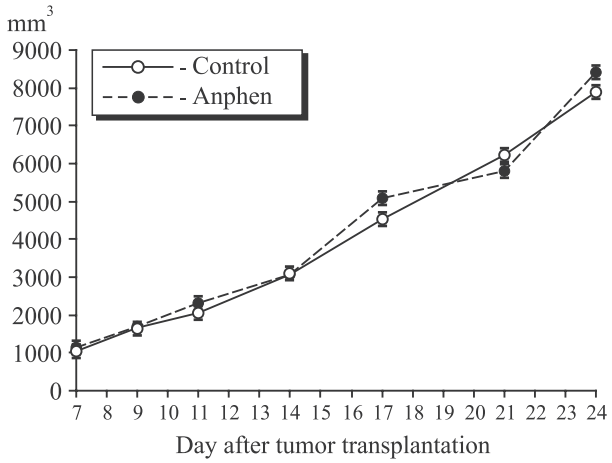

Fig. 3. Kinetic curves of Lewis carcinoma growth in the control and after administration of 1 mg/ml anphen.

depending on the administration route and concentration of the drug. The previously detected 100% inhibition of the growth of sarcoma-37 with anphen after its 11-fold intraperitoneal injection in doses of 10 and 100 mg/kg into the tumor after transplantation can be explained by enhanced apoptosis of intraperitoneal ascitic tumor cells.

The method of blood testing used in this work can be used for tracing the development of the tumor process and for evaluation of antitumor activity of various physicochemical agents.

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