7. S. N. Kuz'mina, T. V. Bul'dyaeva, and I. B. Zbarskii, Biokhimiya, No. 8, 1417 (1980).
8. A. V. Peskin, I. B. Zbarskii, and A. A. Konstantinov, Biokhimiya, No. 4, 579 (1981).
9. N. V. Éngel'gardt, A. I. Gusev, and A. I. Poltoranina, Byull. Éksp. Biol. Med., No. 9, 87 (1972).
10. F. M. Davis, F. Gyorkey, R. K. Busch, et al., Proc. Natl. Acad. Sci. USA, 76, 892 (1979).
11. D. Y. Mason, C. Farrel, and C. R. Taylor, Br. J. Haematol., 31, 361 (1975).
12. L. J. Old, Cancer Res., 41, 361 (1981).
13. J. B. Preer, J. Immunol., 77, 52 (1956).
14. K. Wakabayashi, S. Wang, and L. S. Hnilica, Biochemistry (Washington), 13, 1027 (1974).
15. T. Weller and A. H. Coons, Proc. Soc. Exp. Biol. (N. Y.), 86, 789 (1954).

MOLECULAR MECHANISM OF THE COMBINED ACTION OF AMPHOTERICIN B AND CYCLOPHOSPHAMIDE ON VIRUS-INDUCED NEOPLASIA

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The effect of enhancement of the selective action of antitumor compounds by antibiotics of the polyene group [5, 7, 11] is generally regarded as the result of increased penetration of the chemotherapeutic agent into the neoplastic cell [15]. The writers showed previously that administration of the polyene antibiotic levorin to rats with Pliss lymphosarcoma in a dose not inhibiting neoplastic growth causes some degree of proteolytic degradation of the tumor chromatin proteins. Combined administration of the polyene and cyclophosphamide potentiates the antitumor action of the cytostatic. Proteolytic degradation of chromatin proteins induced by levorin is potentiated under these circumstances (administration of cyclophosphamide alone does not change the fractional composition of the tumor chromatin proteins) [4]. These observations indicate that when administered in conjunction with cytostatics, the polyene antibiotic can not only increase the permeability of the cell membranes for cytostatics but can also essentially damage the chromatin proteins.

Since experience in clinical oncology [12, 14] and experiments on animals with tumors [6, 10] have clearly demonstrated the prospects for the use of amphotericin B for potentiating the specific antitumor action of various alkylating preparations, the investigation described below was carried out to study the molecular mechanism of this phenomenon on models of virus-induced neoplasia.

EXPERIMENTAL METHOD

Experiments were carried out on 9-11-day chick embryos and 12-day-old chicks of the Russian White breed with C/O phenotype belonging to the VNIIRGZh "leukemia-free" cross line. Contamination of the birds with viruses of the avian leukemia-sarcoma group was monitored by the complement fixation test, indirect hemagglutination test, and pathomorphologically. The tumor was induced by Rous sarcoma viruses (RSV) belonging to different serological groups, using strains RSV (RAV-1), RSV (RAV-2), RSV (RAV-49), and RSV (RAV-50). The titer of viruses was $10^{-4.5}$-$10^{-6.5}OD_{50}$/0.1 ml.* The chicks were divided into four groups. In the special experimental group, 3 h after inoculation of the virus amphotericin B was given by mouth in a dose of 2 mg, dissolved in 1 ml water, and 1 h later 10 mg/kg cyclophosphamide in physiological saline was injected intraperitoneally. Chicks of the three control groups received one of the preparations, respectively: amphotericin B — group C_p, cyclophosphamide — group C_c, and

*OD: oncogenic dose.

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TABLE 1. Effect of Combined Administration of Amphotericin B and Cyclophosphamide on Growth of Tumors Induced by Rous Sarcoma Virus

Strain of virus	Group	Number of chicks in group	Inhibition of tumor growth		Weight of tumor, g
			total number of chicks	%	
RSV (RAV-1)	C_o	40	—	—	16,4±1,3
	C_c	37	17	45,9	12,6±0,9
	C_p	35	14	40,0	13,5±1,1
	Experimental	37	22	59,4	9,3±0,7 ($P<0,05$)*
RSV (RAV-50)	C_o	29	—	—	18,2±1,7
	C_c	25	9	36,0	15,9±1,0
	C_p	25	8	32,0	16,3±1,2
	Experimental	26	14	53,8	11,2±1,3 ($P<0,01$)*

*Compared with group C_c.

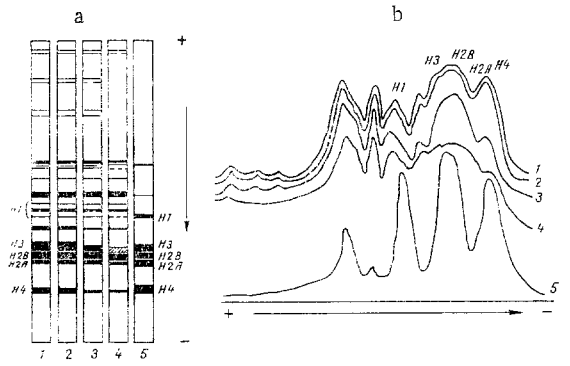

Fig. 1

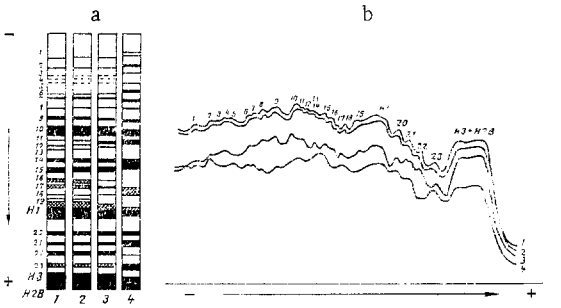

Fig. 2

Fig. 1. Schemes (a) and densitograms (b) of gels after electrophoresis of acid-soluble Rous sarcoma chromatin proteins isolated from chicks of four experimental groups. 1) C_o; 2) C_c; 3) C_p; 4) experimental group; 5) calf thymus.

Fig. 2. Schemes (a) and densitograms (b) of gels after electrophoresis of total Rous sarcoma chromatin proteins, isolated from chicks of four experimental groups. Legend as to Fig. 1.

the solvent — group C_o. Chicks of all groups received daily injections of the preparations for 5 days, and on the 10th day they were exsanguinated and the tumors removed. The experiments were repeated at least three times. Chromatin was isolated by washing the tissue with 0.075 M NaCl solution containing 0.024 M EDTA-Na (pH 6), using a nonpolar detergent at one stage of isolation [8]. Water-soluble cytoplasmic proteins (supramitochondrial supernatant) were obtained by centrifugation of the supernatant obtained in the first stage of chromatin isolation for 1 h at 20,000g. Acid-soluble proteins were extracted from chromatin with 0.2 M H_2SO_4; histone fraction H1, for identification in the composition of the acid-soluble chromatin proteins, was isolated by stepwise precipitation with TCA. Electrophoretic fractionation of total chromatin proteins in 10% polyacrylamide gel in the presence of sodium dodecylsulfate was carried out by the method described in [4]. Acid-soluble proteins were separated in the usual way [13]. The amphotericin B used in the experiments was supplied by the Kurgen "Sintez" Medical Preparations Combine.

EXPERIMENTAL RESULTS

A series of preliminary experiments showed that amphotericin B inhibits the development of foci of neoplastic transformation of chorioallantoic membrane (CAM) cells of developing chick embryos by 65-70%. The effect was more marked when the preparation was given 1 h before inoculation of the virus-containing material.

After peroral administration of the polyene antibiotic to chicks in a dose of 0.25 MPD_{50}, the antiviral and antitumor effect was 25-30% below that in experiments *in ovo*. If the antibiotic was injected directly into the tumor, there was no effect.

The antitumor activity of the cytostatic injected in the maximal tolerated concentration was commensurate with the action of the polyene, but varied depending on the strain of RSV used. The "strain" sensitivity of tumors induced by RSV to the alkylating agent was distributed in the following order: RSV (RAV-1) = RSV (RAV-2) > RSV (RAV-49) ≥ RSV (RAV-50).

Depending on the strain of RSV used, amphotericin B potentiated the antitumor action of cyclophosphamide (Table 1).

The fractional composition of the acid-soluble chromatin proteins is shown in Fig. 1. The histone H1 fraction consisted of three components; the other histone fractions agreed well in their electrophoretic mobility with histones from calf thymus chromatin, taken for comparison. Besides histone proteins, 12 nonhistone components also were present in the acid-soluble chromatin proteins. Injection of cyclophosphamide did not affect the fractional composition of the acid-soluble proteins. Under the influence of amphotericin B there was a small decrease in the relative content of histone fraction H1 and a significant decrease in the level of fraction H4. It was shown previously that the former is connected with activation of cytoplasmic proteases by the polyene antibiotic [4], whereas the latter is due to extraction of the H4 histone fraction by sodium deoxycholate, which is added to the pharmaceutical form of amphotericin B [3]. Combined administration of the preparations led to a greater fall in the relative content of the H1 histone fraction, which is most sensitive to the action of proteases, than in the C_p group and to a decrease in the relative content of some of the acid-soluble nonhistone proteins. This trend of the changes was evidently connected with an increase in protease activity during combined use of the two preparations.

During fractionation of total chromatin proteins the most mobile fractions of the nonhistone proteins were located between histone fractions H1 and H3, so that the length of the gels could be increased by continuing the fractionation for 19 h. As Fig. 2 shows, in group C_c the fractional composition of nonhistone proteins was unchanged compared with group C_o. Administration of amphotericin B caused qualitative and quantitative shifts among the nonhistone proteins with average electrophoretic mobility. Combined administration of the preparations potentiated these changes sharply, and the effect spread to the region of low electrophoretic mobilities; in the region of highly mobile nonhistone proteins (between histone fractions H1 and H3) significant quantitative changes were observed.

Comparison of the fractional compositions of cytoplasmic proteins of Rous sarcoma cells by electrophoresis in the presence of sodium dodecylsulfate demonstrated their complete identity for all four experimental groups of chicks. This was probably connected with the resistance of the globular protein structures to the action of proteases.

The main target for the cytotoxic action of alkylating compounds is nuclear DNA [1, 9]. Its accessibility for DNA-tropic substances is determined by chromatin proteins, of which the most important are histones of the H1 fraction [2]. Damage to this histone fraction after combined administration of amphotericin B and cyclophosphamide, demonstrated in the present investigation, indicates increased accessibility of tumor cell DNA to the action of the alkylating agent.

The results obtained on models of virus-induced tumors agree largely with the results of investigations carried out previously on transplantable tumors [4]. This demonstrates the universal character of the molecular mechanisms of the combined action of polyene antibiotics and alkylating agents on the development of the neoplastic process irrespective of its etiology.

LITERATURE CITED

1. A. K. Belousova, in: Chemotherapy of Malignant Tumors [in Russian], Moscow (1977), pp. 61-117.
2. G. P. Georgiev and V. V. Bakaev, Mol. Biol., No. 6, 1205 (1978).
3. L. S. Kravchenko, Antibiotiki, 22, 233 (1977).
4. A. Ya. Oksman, M. A. Shneider, N. K. Asinovskaya, et al., Éksp. Onkol., 3, No. 4, 37 (1981).
5. M. A. Shneider, in: Advances in the Study and Production of Antibiotics [in Russian], No. 6, Moscow (1979), p. 56.
6. M. A. Shneider, A. A. Eremeeva, L. A. Rachkovskaya, in: Current Problems in Experimental Tumor Chemotherapy [in Russian], Vol. 2, Chernogolovka (1980), p. 95.
7. K. V. Yaremenko, A. A. Eremeeva, and I. M. Tereshin, Antibiotiki, 22, 153 (1977).

8. K. M. Anderson, H. Chance, and N. Kadohama, Exp. Cell. Res., 94, 176 (1975).
9. T. A. Connors, in: Cancer Chemotherapy, Amsterdam (1979), p. 25.
10. G. Lorent, J. Bewerie-Vanhouche, D. Machin, et al., Cancer Res., 40, 939 (1980).
11. G. Medoff, D. Schlessinger, and G. S. Hebayashi, Cancer Res., 34, 823 (1974).
12. I. Nakasawa, F. Ouchi, and N. Ishida, J. Exp. Med., 131, 101 (1980).
13. S. Panium and R. Chalkley, Biochim. Biophys. Acta, 130, 337 (1969).
14. C. A. Presant, S. Hillinger, and C. Klahr, Cancer (Philadelphia), 45, 6 (1980).
15. F. Valerioto, G. Medoff, and J. Diechman, Cancer Res., 39, 2041 (1979).