CHANGES IN COMPOSITION OF DNA-BOUND LIPIDS OF SARCOMA 37 INDUCED BY SARCOLYSIN IN VIVO

V. A. Struchkov and N. V. Strazhevskaya

UDC 615.277.3.015.4:616-006.3.04-008.915-092.9

Key Words: sarcoma 37; sarcolysin; DNA-bound lipids.

An important role in the molecular mechanism of the cytotoxin action of various antitumor compounds is ascribed [11] to structural injuries in DNA (ruptures, DNA—DNA and DNA—protein cross-linkages). However, this problem is not limited to these injuries, for supramolecular levels of DNA organization exist in the chromatin of eukaryotes, in whose function an important role belongs to nonhistone proteins (NHP), RNA [2, 9, 10], and also tissue-specific DNA—bound lipids [1, 9, 13-15], which are easily and irreversibly damaged by γ-ray irradiation of the animals [9, 14].

The aim of this investigation was to study the composition of DNA—bound lipids of sarcoma 37 under normal conditions and under the influence of sarcolysin — (p-di-2-chloroethyl)-amino-D,L-phenylalanine — in order to come closer to an understanding of the mechanism of action of this bifunctional alkylating agent, which is extensively used in clinical practice.

EXPERIMENTAL METHOD

Cells of ascites sarcoma 37 of mice were used as the test object. Sarcolysin in a solution of 0.14 M NaCl was injected intraperitoneally once in the maximally allowable dose (MAD, 10 mg/kg) into noninbred male mice weighing 30-35 g on the 7th day after inoculation. The mice were decapitated 5 h after injection of sarcolysin, and the control mice received an aliquot of 0.14 M NaCl.

DNA was isolated by the phenolic method [4, 10], modified so that the DNA could be extracted in two fractions: the basic fraction I (60%) — water-soluble supramolecular DNA complex (SMC DNA), containing 2-3% of NHP, and according to [7], consisting of circular structures; fraction II (40%) — "residual" DNA on the phenol—water phase boundary, containing the replicative fork [3] and very rich in firmly bound NHP (15-25%) [3, 9], containing a group of proteins of the nuclear matrix [12], and which we called DNA of the "phenolic" nuclear matrix (PNM DNA). SMC DNA was obtained after the 4th phenolization with a mixture of 66% of water-saturated phenol, pH 8.5, and 0.14M NaCl (1:1), including three treatments of SMC DNA together with PhM DNA and a single treatment without it. The PhM DNA was collected after the third phenolization, washed twice with the above-mentioned mixture on centrifugation, and resuspended in 10 ml of 0.14 M NaCl. Phenol was removed by dialysis.

The DNA content was measured with dephenylamine and the elastic viscosity of the SMC DNA measured as in [10]. Extraction, fractionation of the lipids by thin-layer chromatography on silica-gel H (from "Merck," West Germany), and determination of the fractions were carried out as described previously [5], but the measurement of the lipid fractions at 375 nm was done on a "Beckman-26" spectrophotometer in cuvettes (1 cm). Since standard extraction of lipids with chloroform—methanol (2:1) removed only some of the lipids from SMC DMA, this suggested that they contained weakly and strongly bound lipids [2, 5]. The SMC DNA was therefore extracted first with 35% ethanol (weakly bound lipids, WBL), and the DNA residue was then incubated With DNAase I (not containing lipids, from "Koch Light," England) in the presence of 0.01 M $MgCl_2$ for 3 h at 37°C (firmly bound lipids — FBL). In the case of PNM DNA, this was treated immediately with DNAase I.

All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR G. A. Zedgenidze.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 108, No. 9, pp. 327-330, September, 1989. Original article submitted April 5, 1988.

 0007-4888/89/0009-1298$12.50

TABLE 1. Composition of Lipids of SMC DNA and PNM DNA of Sarcoma 37 under Normal Conditions and 5 h after Injection of Sarcolysin ($M \pm m$)

Lipids	Supramolecular complex of DNA							
	control						experiment	
	WBL		FBL		total L		WBL	
	μg*	%**	μg	%	μg	%	μg	%
FC	15,3±1,5	10,7	11,2±1,1	19,8	26,5±2,3	13,3	30,9±2,8	13,4
DG	27,1±2,5	18,8	14,8±1,5	26,3	41,9±4,0	21,1	32,3±3,1	14,0
FFA	31,0±3,0	21,6	15,3±1,3	27,3	46,4±4,1	23,3	42,4±3,9	18,4
TG	4,9±0,6	3,4	2,3±0,3	1,8	7,2±0,8	3,1	7,0±0,6	3,0
CE	65,2±6,1	45,5	12,8±1,1	22,8	78,0±7,2	39,2	118,0±15,0	51,2
NL	143,5±12,0	100	56,5±5,5	100	200,0±17,3	100	230,6±21,0	100
PI	0,6±0,1	1,4	3,2±0,4	12,0	3,8±0,4	5,8	6,5±0,6	5,5
PC + PS	9,7±0,9	25,7	5.5±0,6	20,5	15,2±1,4	23,4	17,8±1,6	14,9
PE	8,6±0,9	22,5	9,3±0,9	34,7	17,9±1,6	27,6	19,2±1,7	16,1
CL	19,3±1,8	50,4	8,7±0,8	32,8	28,0±2,4	43,2	75,8±6,1	63,5
PL	38,2±3,6	100	26,7±2,3	100	64,9±5,7	100	119,3±9,5	100
NL/PL	3,75		2,11		3,08		1,93	
CE/FC	4,26		1,15		2,95		3,81	
C/PL	4,25		1,74		3,26		2,54	

Lipids	Supramolecular complex of DNA				PNM DNA			
	experiment				control		experiment	
	FBL		total L		total lipids			
	μg	%	μg	%	μg	%	μg	%
FC	15,2±1,4	19,6	46,2±4,3	15,0	18,4±1,6	18,7	28,6±2,6	37,3
DG	23,7±2,5	30,7	56,0±5,2	18,2	29,6±3,0	30,1	11,3±1,2	14,7
FFA	20,9±1,8	27,0	63,3±5,0	20,5	24,3±2,0	24,7	20,4±1,7	26,6
TG	4,4±0,5	5,7	11,4±1,0	3,7	1,8±0,2	1,8	2,9±0,3	3,8
CE	13,1±1,2	16,9	131,1±12,0	42,6	24,3±2,1	24,7	13,5±1,2	17,6
NL	77,3±7,1	100	308,0±21,1	100	98,4±7,5	100	76,7±6,1	100
PI	3,9±0,4	6,6	10,4±1,1	5,8	2,9±0,3	8,2	0	0
PC + PS	16,0±1,4	27,3	33,8±3,1	19,0	13,1±1,2	37,4	24,7±2,1	72,5
PE	17,2±1,5	29,3	36,4±3,1	20,4	9,8±0,8	28,0	4,9±0,5	14,5
CL	21,6±1,9	36,8	97,4±8,0	54,8	9,2±0,8	26,4	4,4±0,5	13,0
PL	58,7±4,9	100	178,0±12,5	100	35,0±2,9	100	34,0±2,5	100
NL/PL	1,36		1,73		2,81		2,25	
CE/FC	0,86		2,84		1,32		0,47	
C/PL	0,97		2,02		2,48		2,52	

Legend. *) μg lipids/10 mg DNA; **) each lipid as percent of total NL or PL, taken as 100.

The results were expressed in μg lipids/10 mg DNA and the percentage composition of the lipids also was calculated, the total of neutral lipids (NL) or phospholipids (PL) being taken as 100%. The results of three experiments in three repetitions were analyzed by Student's t test.

EXPERIMENTAL RESULTS

The characteristics of the lipid components of SMC DNA and PNM DNA of sarcoma 37 under normal conditions and after treatment with sarcolysin are given in Table 1. Clearly SMC DNA of sarcoma 37 contained a minor, but constant quantity of neutral (200 ± 17) and polar lipids (64.9 ± 5.7), calculated per 10 mg DNA. NL consisted of three main fractions: cholesterol esters (EE, 39%), free fatty acids (FFA, 23%), and diglycerides (DG, 21%), and also a minor fraction of free cholesterol (FC, 13%) and traces of triglycerides (TG, 3%). PL consisted mainly of cardiolipin (CL, 43%) and phosphatidylethanolamine (PE, 27%), but they were poor in the fraction of phosphatidylcholine with phosphatidylserine (PC + PS, 23%) and contained a minor fraction of phosphatidylinositol (PI, 6%).

It is interesting to note that PNM DNA contained only half the amount of neutral and polar lipids contained in SMC DNA, and in rather different proportions. For instance, its neutral lipids were richer in DG (30%) and FC (19%) but poorer in CE (25%), and among the phospholipids, the dominant fraction was PC + PS (37%), and the proportion of CL was considerably reduced (26%). On the whole the distinguishing feature of the lipids in the two DNA fractions was the considerable preponderance of NL over CL, evidence of their intranuclear origin.

The lipid components of SMC DNA of sarcoma 37 was shown to consist of FBL and WBL, which differ in their composition. For instance, for FBL the quantity of nearly all fractions was specifically increased by 1.5-3 times, especially FC, CE, FFA, CL, and PI. As a result, the basic parameters, i.e., the ratios NL/PL, CE/FC, and C/PL were considerably higher in WBL than in FBL, confirming our hypothesis [2] that their role differs in the structural and functional organization of SMC DNA of eukaryote cells.

A characteristic feature of the lipid components of SMC DNA of sarcoma 37 by contrast with SMC DNA of normal eukaryote cells [15] is the presence of a minor TG fraction, and also the equal values of three basic parameters such as CE/FC, NL/PL, and C/PL. The latter vary considerably (by 2-3 times) in normal cells [15]. Moreover, sarcoma 37 also is characterized by the fact that PNM DNA accounts for a high percentage of the total DNA of the nucleus (40% compared with 1-3% in rat thymus and liver), which is typical also of fibroblasts undergoing malignant change, as the writers showed previously [6]. It can be tentatively suggested that during malignant transformation the number of sites of attachment of DNA to the nuclear matrix (membrane) is increased and (or) the character of these sites changes. The fact that the composition of FBL in SMC DNA closely resembles the composition of the lipids in DNA suggests that they are residual matrix lipids in SMC DNA. Other evidence in support of this view is given by the presence of endogenous RNA polymerase II in SMC DNA [15], which is firmly bound with DNA of the nuclear matrix [8].

It was shown that 5 h after treatment with sarcolysin considerable changes took place in the quantity and quality of lipids in the DNA fractions. For instance, the quantity of total NL in SMC DNA was increased by 1.5 times and the quantity of PL by 3 times. However, an opposite and weaker effect is observed in FNM DNA, and only at the level of total NL. A result of the redistribution, induced by sarcolysin, within the lipid components of SMC DNA is a sharp decrease (by 1.5 times) in the NL/PL and C/PL ratios, whereas the decrease is smaller in PNM DNA. Analysis at the WBL and FBL levels in SMC DNA showed that sarcolysin causes an increase in the total NL and PL contents in the two components, but the effect is more marked in WBL, in which case the greatest contribution is made by FC, CE and, in particular, by CL and PI.

Thus sarcolysin, in MAD in vivo, causes considerable redistribution in the composition of the DNA-bound lipids of sarcoma 37, manifested in the case of SMC DNA by a sharp rise in the content of all fractions of NL and PL (especially CE, FC, CL, and PI) and, conversely, in the case of PNM DNA by a sharp decrease in the content of CL, PE, and DG and disappearance of PI, with at the same time an increase in FC, TG, and PC + PS. Meanwhile, sarcolysin reduced by 34% the elastic viscosity of SMC DNA (η_{sp}/C: control 397 ± 43, experiment 264 ± 31).

To conclude, DNA-bound lipids may be the target for the action of sarcolysin, just as for γ-radiation [9, 14]. Moreover, the specificity of DNA-bound lipids which we found, and which is characteristic of sarcoma 37 (the presence of TG and a high content of CL and CE), may be used to create lipid-cytostatics with selective action on tumor cells.

LITERATURE CITED

1. A. V. Alisenko, E. B. Burlakova, and É. A. Pantaz, Biokhimiya, **49**, 621 (1984).
2. S. D. Belyaev, N. B. Strazhevskaya, I. K. Kolomiitseva, et al., Dokl. Akad. Nauk SSSR, **214**, No. 5, 1189 (1974).
3. V. S. Dashkevich, A. P. Kuz'menko, N. A. Skobeleva, et al., Dokl. Akad. Nauk SSSR, **270**, No. 3, 1236 (1983).
4. G. P. Georgiev and V. A. Struchkov, Biofizika, **5**, No. 6, 745 (1960).
5. Z. I. Krasichkova and N. V. Strazhevskaya, Radiobiologiya, **21**, No. 2, 172 (1981).
6. S. V. Kuz'mina and N. V. Strazhevskaya, Biofizika, **15**, No. 6, 1133 (1970).
7. V. D. Paponov, O. B. Fabrichnaya, and I. A. Kuznetsov, Mol. Biol., **18**, No. 6, 1533 (1984).
8. S. V. Razin, O. V. Yarovaya, and G. P. Georgiev, Dokl. Akad. Nauk SSSR, **281**, No. 1, 220 (1985).
9. N. V. Strazhevskaya and V. A. Struchkov, Radiobiologiya, **17**, No. 2, 163 (1977).
10. V. A. Struchkov, Biofizika, **7**, No. 5, 538 (1962).
11. V. A. Struchkov, Current Problems in Experimental Tumor Chemotherapy [in Russian], Vol. 1, Chernogolovka (1980), p. 122.
12. V. A. Struchkov, D. Yu. Blokhin, and N. V. Strazhevskava, Structure and Functions of the Cell Nucleus [in Russian], Chernogolovka (1987), p. 130.

13. F. A. Manzoli, S. Capitani, N. M. Maraldi, et al., Adv. Enzyu. Reg., **17**, 175 (1979).
14. N. V. Strazhevskaya, V. A. Struchkov, et al., Stud. Biophys., **53**, No. 1, 73 (1975).
15. N. V. Strazhevskaya, Z. I. Krasichkova, and N. L. Kruglova, Stud. Biophys., **76**, No. 3 (1979).

INDUCTION OF TUMORICIDAL ACTIVITY OF HUMAN AND MURINE PERITONEAL MACROPHAGES BY ANTITUMOR CHEMOTHERAPY

S. L. Potapov and M. M. Vyadro

UDC 616-006-02:615.277.3]07:616+381-008.853.3

Key words: macrophages; cytotoxicity; tumor cells; cytostatics.

Cytostatics used in the tumor chemotherapy are traditionally related to immunodepressants. Recently the heterogeneity of action of chemotherapeutic agents on the immune system has been demonstrated. For instance, the immunomodulating effects of alkylating agents (cyclophosphamide, melphalan), antimetabolites (mercaptopurine, 5-fluorouracil), antitumor antibiotics (doxorubicin, bleomycin), plant alkaloids (vinblastine, vincristine), platinum compounds, etc., have been discovered [1, 4, 6]. There is evidence that cytostatics can activate cells of the mononuclear phagocytic system [3, 4, 5, 7]. It has been shown, in particular, that the presence of macrophages potentiates the cytotoxic action of chemotherapeutic drugs on tumor cells cultured in agar diffusion chambers [2].

The aim of this investigation was to study the ability of cytostatics (platidiam, cyclophosphamide, 5-fluorouracil, adriamycin, and aclarubicin) to induce tumoricidal activity of human and murine peritoneal macrophages (PM).

EXPERIMENTAL METHOD

Human peritoneal cells were isolated from ascites fluid obtained from patients with disseminated ovarian carcinoma, during therapeutic paracentesis. The cells were washed and suspended in medium RPMI-1640 with 10% fetal serum, glutamine (2 mM), HEPES (10 mM), and gentamicin (50 μg/ml). The cells were counted, their viability determined (with the aid of 0.1% trypan blue solution), after which they were introduced into flat-bottomed wells of 96-well panels at the rate of $5 \cdot 10^5$ PM per well. After incubation for 2 h at 37°C in 5% CO_2 the monolayer of adherent cells (autologous tumor-associated PM – ATPM) were vigorously washed and incubated for the next 18 h with the chemotherapeutic agents or with medium (control), after which the adherent cells were again washed and used as effectors. Nonadherent cells (autologous tumor target cells – ATTC) were collected and cultured for 18 h under the same conditions as the PM, after which they were added to the ATPM in the ratio of 1:5-1:50. The ATPM were incubated with ATTM for 48 h, after which the number and viability of the ATTM were determined with 0.1% trypan blue solution. The cytotoxic index (CTI) was calculated by the formula:

$$\text{CTI} = (A/A_1 - B/B_1) \cdot 100\%,$$

where A and B denote the number of living tumor cells in the wells after culture in the presence of macrophages (B) and in their absence (A); A_1 and B_1 denote the initial number of cells.

To obtain murine PM the peritoneal cavity of DBA/2 and (C57BL/6 × DBA/2)F_1 mice (five mice were used in each variant of the experiment) was washed with cold (on ice) medium 199 with 10% fetal serum, heparin (10 U/ml), and monomycin (20 U/ml). The peritoneal cells were washed 3 times with the same medium (without heparin), centrifuged, counted, made up to a concentration of $2 \cdot 10^6$ PM/ml, and transferred in a volume of 0.2 ml ($4 \cdot 10^5$ PM/well) into flat-bottomed wells of panels. The subsequent stages of obtaining the PM monolayer and its processing were the same for

Institute of Chemical Physics, Academy of Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR S. M. Navashin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 108, No. 9, pp. 330-332, September, 1989. Original article submitted March 15, 1988.

