DISTURBANCE OF TUMOR CELL DNA REPAIR BY SARCOLYSIN

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The bifunctional alkylating agent sarcolysin — β-p-di-(2-chloroethyl) aminophenyl-α-alanine hydrochloride — is a preparation with high selectivity and a broad spectrum of antitumor action.

Its synthesis was based on the idea of Academician L. F. Larionov that unsubstituted metabolites (in this case the amino acid phenylalanine) will guide the alkylating cytotoxic group predominantly into tumor cells. Sarcolysin was the first Soviet preparation of this kind, which when administered to animals in the highest tolerated single dose caused complete absorption of several transplantable tumors, accompanied by a moderately toxic action on normal tissue [2]. Sarcolysin is nowadays used widely in clinical practice for the treatment of multiple myeloma, carcinoma of the ovaries, seminoma, and other malignant tumors [3]. According to data in the literature sarcolysin has a complex mechanism of action: it disturbs the energy metabolism of tumor cells and exhibits antimetabolite properties at the cell membrane level. Meanwhile, like other chloroethylamines, it can alkylate nucleic acids and nucleoproteins, can form DNA-DNA and DNA-protein cross-linkages, and can induce breaks in DNA molecules [1].

To study the alkylating activity of sarcolysin, dependence of the formation of defects in DNA molecules of tumor cells, namely cross-linkages and single-strand breaks arising under the influence of the compound, on time and dose was investigated. Another aim of the investigation was to study preparative DNA synthesis in cells treated with sarcolysin.

EXPERIMENTAL METHOD

Noninbred male mice weighing 20-25 g with transplantable ascites sarcoma 37, adapted to growth in vitro, were used. Experiments were carried out on suspension cultures of tumor cells. The culture medium contained: medium 199 and Eagle's medium, bovine serum (in the ratio of 7:3:1), 10,000 U of streptomycin, and 0.6 ml of 3% glutamine to 100 ml of medium. The number of cells was counted in a Goryaev's chamber and adjusted to 5×10^5 in 1 ml. The samples were incubated at 37°C with ^{3}H-thymidine (specific activity TB_q* mole, 37 kBq per sample) for 2-18 h depending on the experimental conditions. The compound was dissolved in physiological saline, added to the samples up to final concentrations of between 1 and 500 μM, and incubated at 37°C for 1, 2, 4, or 18 h. After incubation the cells were washed to remove excess radioactivity by centrifugation at 4000 rpm for 2 min on the TsLN centrifuge, suspended in 1 ml of physiological saline, and added to 1 ml of lytic solution (0.6 M NaOH, 0.2 M Na_2-EDTA, 0.25% sodium dodecylsulfate). Lysis was carried out at room temperature for 1 h. The lysates were removed from the samples by means of an automatic pipet with a metal tip (length 10 m, diameter 2.5 mm), which led to partial mechanical fragmentation of DNA due to hydrodynamic shearing [5]. The lysates were investigated by zonal ultracentrifugation in a 5-20% alkaline sucrose density gradient, containing 0.2 M NaOH, 0.8 M NaCl, and 0.02 M Na_2EDTA. The lysate, in a volume of 0.3 ml (1.2 μg DNA), was layered above the gradients and centrifuged for 3 h at 25,000 rpm (20°C) on a Beckman (USA) centrifuge, in the SW-27 rotor (6 × 13.2 ml). The gradients were divided into fracitons with the aid of an automatic syphon, and these were then neutralized by the addition of 1 N HCl.

*No value given in Russian original — Publisher.

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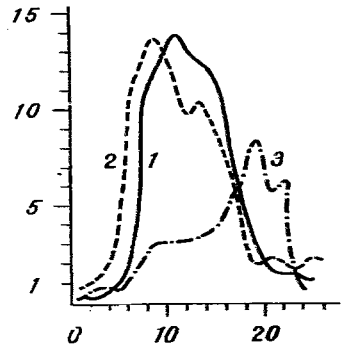

Fig. 1

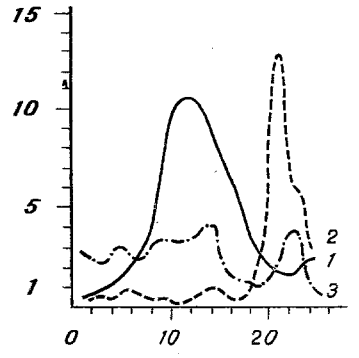

Fig. 2

Fig. 1. Sedimentation diagrams of single-stranded DNA in a 5-20% alkaline sucrose density gradient. Here and in Figs. 2 and 3: abscissa, No. of fractions (left – bottom, right – meniscus); ordinate, radioactivity (in dis/min × 10^3). 1) Control; 2) 1 μM sarcolysin; 3) 10 μM sarcolysin. Duration of incubation with sarcolysin 2 h.

Fig. 2. Sedimentation diagrams of single-stranded DNA in 5-20% alkaline sucrose density gradient. 1) Control; 2) 250 μM sarcolysin; 3) 500 μM sarcolysin. Duration of incubation with sarcolysin 18 h.

TABLE 1. Increase in Number of Single-Strand Breaks in DNA Molecules from S-37 Cells under the Inlfuence of Sarcolysin

Concentration of sarcolysin, μM	Incubation time, h		
	2	4	18
20	0,5—1	0,5—2	7—8
100	0,7—1,2	1,3—1,7	8—9
250	D	0,2	15,4
500	0,25	D	27,5

Legend. D) Degradation of DNA.

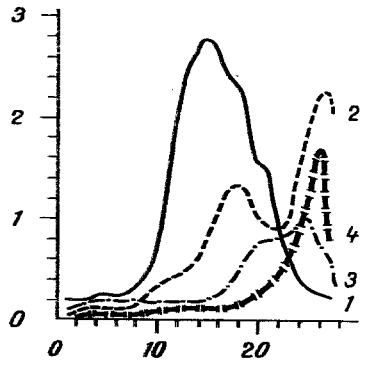

Fig. 3. Sedimentation diagrams of single-stranded DNA in 5-20% alkaline sucrose density gradient. 1) Control; 2) 10 μM sarcolysin (incubation for 1 h); 3) 5 mM HU (preincubation for 15 min); 4) 5 mM HU (preincubation for 15 min) and 10 μM sarcolysin (incubation for 1 h). Duration of incubation after change of medium was 18 h.

Radioactivity was determined in ZhS-8 scintillator on a Nuclear Chicago Mark III (USA) counter. The results were presented in the form of sedimentation diagrams. The average molecular weight was determined from a table [6] and by a formula [8].

For a detailed study of reparative DNA synthesis it was necessary to remove the preparation from the culture medium and to uncouple replication and repair of DNA. For this purpose, cells treated with sarcolysin (1-2 h) were washed to remove the agent, the incubation medium was changed, after which the samples were incubated for 2-18 h at 37°C. Hydroxyurea (HU), which was added to the experimental samples up to a final concentration of 5 mM in 15 min before the addition of ^{3}H-thymidine, was used as the inhibitor of DNA replication. The cells were pulse-labeled with ^{3}H-thymidine for 1 h (185 kBq per sample).

EXPERIMENTAL RESULTS

Sedimentation diagrams of DNA from intact tumor cells, after centrifugation in an alkaline sucrose density gradient under the conditions described above, revealed a distinct peak with mol. wt. of about (1.8-2.0) × 10^8 daltons.

The time course of appearance of defects in the structure of the DNA molecules under the influence of sarcolysin was studied in the experiments of series I. Sarcolysin was shown to have a cross-linking action on DNA in a relatively low concentration (1 μM) actually during the first 1-2 h of incubation with the tumor cells (Fig. 1, 2). An increase in the sarcolysin concentration to 10 μM led to the rapid disappearance of the cross-linkages and to intensive accumulation of label in the low-molecular-weight region of the sedimentation diagram, proportionally to the incubation time (Fig. 1: 3), indicating the appearance of breaks in the DNA molecules. This fact is evidence of induction of enzymes of reparative synthesis and the onset of the first phase of the excision repair process — recognition of the region of DNA damaged by the alkylating agents and of nicking of the polynucleotide chain close to the injury, with the participation of specific endonucleases and glycosylases [7].

After incubation with sarcolysin for 18 h profound degradation of DNA was observed and the number of single-stranded breaks reached 7-8 to an original DNA fragment with mol. wt. of 2 × 10^8 daltons (Table 1). This corresponds to about 175,000 breaks per cell. The phenomenon observed can be explained by the onset of the second phase of excision repair, which consists of removal of damaged regions of DNA and its degradation by exonucleases.

However, for a long time during incubation of the cells with high concentrations of sarcolysin (250 or 500 μM, 18 h) no high-molecular-weight peak of radioactivity was seen to appear, and this indirectly pointed to depression of the synthetic phase of repair, connected with filling in of the breaches arising in the DNA molecules by means of polymerases and restorations of the saccharophosphatide bond by polynucleotide ligase, by sarcolysin (Fig. 2: 2, 3).

In the experiments of series II changes in the structure of DNA were investigated after pulsed administration of sarcolysin. After treatment of the cells with sarcolysin for 1 h in a low concentration (1 μM), followed by washing to remove the drug and transfer of the cells to fresh medium for incubation for 18 h, the peak of radioactivity was found to coincide with the control. Low sarcolysin concentrations evidently do not inhibit enzymes of reparative synthesis sufficiently deeply. After the sarcolysin concentration was increased to 10 μM and the medium changed, incorporation of ^{3}H-thymidine did not reach the control level and two peaks appeared on the sedimentation diagram, corresponding to DNA fragments with different molecular weights (Fig. 3: 2).

To separate replication and repair processes, HU was used as an inhibitor of replicative DNA synthesis. Preincubation of the cells with HU in a concentration of 5 mM for 15 min followed by a change of medium led to inhibition of replicative DNA synthesis (Fig. 3: 3), and the sedimentation diagram of DNA from cells treated beforehand with sarcolysin (10 μM) consisted of one peak of radioactivity, shifted into the low-molecular-weight region (Fig. 3: 4). The appearance of a high-molecular-weight peak (Fig. 3: 2) was linked with incorporation of ^{3}H-thymidine into the structurally restored DNA of some of the cells exposed to the action of sarcolysin, or cells not damaged by the drug. The low-molecular-weight DNA fragments observed [mol. wt. about (0.55-1.0) × 10^6], like Okazaki fragments [7], can serve as intermediates for reparative synthesis (Fig. 3: 4).

The data are thus evidence that sarcolysin has an inhibitory action on the synthetic phase of the DNA repair process. The depth of inhibition is dose-dependent. The ability of sarcolysin to induce a rapidly increasing number of irreparable breaks in DNA molecules, disturbing its template function, may perhaps also be responsible for its high antitumor activity, which may be exhibited after a single administration of the maximally tolerated dose.

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INDUCTION OF MICROFILAMENT BUNDLES IN INTERPHASE NUCLEI OF RAT NEURINOMA CELLS BY DIMETHYL SULFOXIDE

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Interest in the study of the biological action of the polyfunctional compound dimethyl sulfoxide (DMSO) on widely different cells of living organisms has recently increased considerably. It is widely used as a cryoprotector, growth stimulator, and chemotherapeutic agent, and is known in medicine as dimexide. A unique property of DMSO is its high transportability. At the same time, it is actively used as an inducer of cell differentiation. The effect of DMSO on cell cultures depends essentially on its concentration, the temperature and duration of action, the degree of differentiation of the cells, and so on [3]. In somatic hybridization experiments DMSO is used as an additional agent which greatly improves the process of cell fusion [9]. In our own experiments on fusion of membranes of tumor cells in the nervous system with the aid of polyethylene-glycol (PEG, mol. wt. 1000) addition of 15% DMSO led to a high yield of polykarya, without any appreciable cytotoxic effect [1]. Meanwhile the morphologic aspects of the action of different DMSO concentrations at the subcellular level have been inadequately studied.

Interest has accordingly increased in the study of the action of a 15% concentration of DMSO, used in fusion experiments, on the ultrastructure of tumor cells, and the investigation described below was undertaken to investigate the problem.

EXPERIMENTAL METHOD

Experiments were carried out on transplantable cell line RGGN-1 obtained from a neurinoma of the rat Gasserian ganglion. Cells of the RGGN-1 line were cultured in Eagle's MEM

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