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A comparative study of the effects of antitumor agents on lysosomes of tumor *in vivo*

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An assumption has been substantiated that therapeutic effects of a number of drugs including antitumor agents are mediated by lysosomal acid hydrolases [1]. Two groups of findings may be considered to favour the suggestion [2]. Firstly, spontaneous tumor regression was associated with an increase in the number and activity of lysosomes in areas of degradation. Secondly, chemotherapy radiation or hormone induced tumor regression in experiment or clinic was accompanied by enhanced acid hydrolase activities in whole tumor or its outlying areas [3–7].

This study aims to clarify how antitumor agents act to influence lysosomes in tumor cells. For this purpose the efficiency of lysosomes was investigated under treatment of tumor bearing mice. The efficiency of lysosomes was appreciated by activity of lysosomal specific enzymes acid phosphotase and DNase. The following antitumor agents were used at therapeutic dose levels: 5-fluorouracile, (5-FU), vinblastine, nitrosomethilurea, thiophosphamide and derivatives of 5-(*N*-symm. triasinil) aminodioxan (N31) and 2-(*N*-symm. triasinil) amino-1, 3-propandiol (N35) (Table 1).

Tumors were taken for study (with no less than 50 per cent regression). Tumor material within every group (8–10 mice) was pooled. Male and female white mice weighing 18–20 g were kept on normal diet *ad lib*. Ascites Ehrlich tumor cells were inoculated intraperitoneally (i.p.) on Day 0 of experiment.

Tumor bearing mice were injected i.p. with 0.2 ml physiological saline or antitumor drug daily for 6–7 days. Treatment was started on day 2 of the experiment. On day 9 (or 10), 18 hr after the last injection the tumor cells were harvested and processed for preparation of lysosomes, soluble fractions or extracts. Lysosomes were isolated as described in [8]. For preparation of soluble fraction the postlysosomal supernatant was centrifuged at 105,000 *g* for 30 min. For preparation of extracts, washed tumor cells were subjected to freezing and thawing three times and centrifuged at 105,000 *g* for 30 min. The results of the study are presented in Table 1.

Acid phosphatase was measured at 25° in a total volume of 1 ml containing 1 μmole *p*-nitrophenilphosphate (NPP), 1 μmole magnesium acetate, 45–50 μmole Tris buffer pH 5.0 and 0.77 M sucrose; protein contents were from 0.6 to 1.2 mg. The reaction was stopped by adding 2 ml 6% NaOH. The activity was expressed in nmole/*p*-nitrophenol (NP) per mg protein for 60 min incubation.

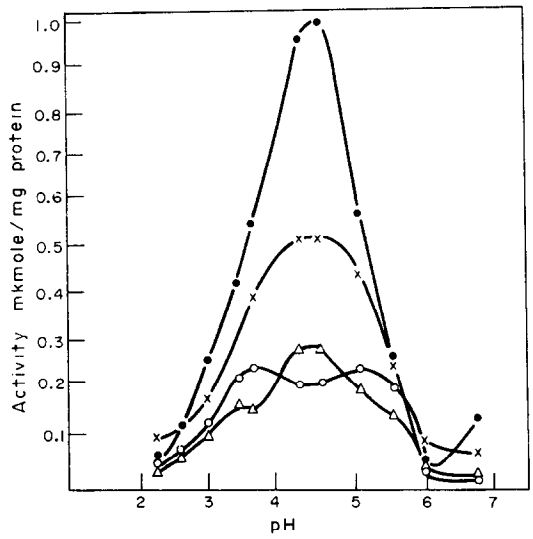

Fig. 1. Acid phosphatase activity in extracts after treatment with various agents.
Abscissa shows pH, ordinates gives activity in nmoles *p*-nitrophenol/mg protein/60 min —— nontreated: O——O N 31: △——△ N 35: ×——× thiophosphamide (activity in extracts treated with 5-FV, vinblastine and nitrosomethylurea was practically equal to that of thiophosphamide).

The DNase activity was estimated by increment in extinction at 260 nm after 20–45 min incubation in a total volume of 3 ml containing 30 T[μ] (30 μg) purified nucleate, 130 nmoles Tris–acetate buffer pH 5.0 and 0.77 M sucrose; protein contents were about 30 μg. The activity was expressed in units per mg protein, 1 unit corresponding to 0.005 increment in extinction. Total activity of the

Table 1. Acid phosphatase and deoxyribonuclease activities (pH 5.0) in lysomes and soluble fraction of ascites Ehrlich carcinoma cells after *in vivo* treatment with antitumor agents

Agents	Doses mg/kg	Acid phosphatase μmoles/mg protein			Deoxyribonuclease units/mg protein		
		Lysosomes		Soluble fraction	Lysosomes		Soluble fraction
		total	free		total	free	
None		0.030	0.011	0.067	61	34	44
5-FU	20	0.056	0.054	0.057	58	57	37
Vinblastine	0.2	0.029	0.027	0.026	56	56	34
Nitrosomethylurea	6.0	0.027	0.027	0.032	61	61	44
Thiophosphamide	3.0	0.027	0.027	0.041	57	55	22
N 31	2.0	0.027	0.027	0.014	56	58	19
N 35	2.0	0.025	0.026	0.016	55	56	15

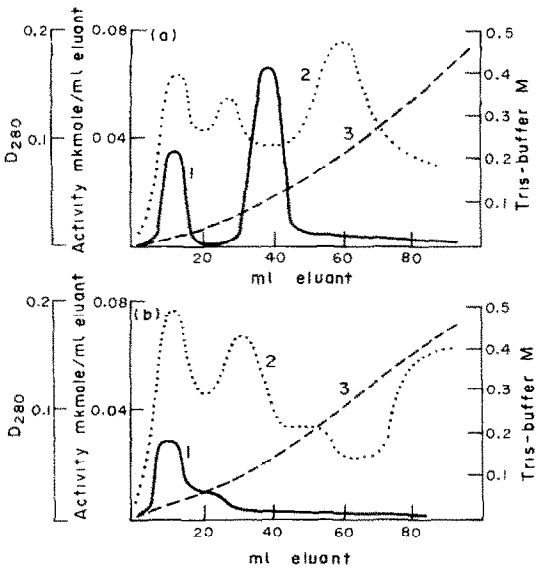

Fig. 2. Isoenzyme patterns of acid phosphatase in extracts of control (a) and treated with N 31 (b) tumors. DEAE-cellulose column chromotography.
(1) activity; (2) E_{280} of eluants; (3) concentration of eluting buffer. Column 5 × 1.2 cm, flow rate 24–26 ml/hr, eluant volumes 4.2–4.5 ml, elution with linear gradient of increasing concentrations of Tris–HCL (0.005–1.0 M, pH 6.3) at 6–7°; activity estimated in a total volume of 1 ml, containing 0.2 ml eluants, 1 μmole *p*-nitrophenilphosphate, 1 μmole magnesium acetate, 450 μmole Tris–acetate, buffer (pH 5.0) as a result of 3 hr incubation at 25° and expressed in μmoles *p*-nitrophenol/1 ml eluant.

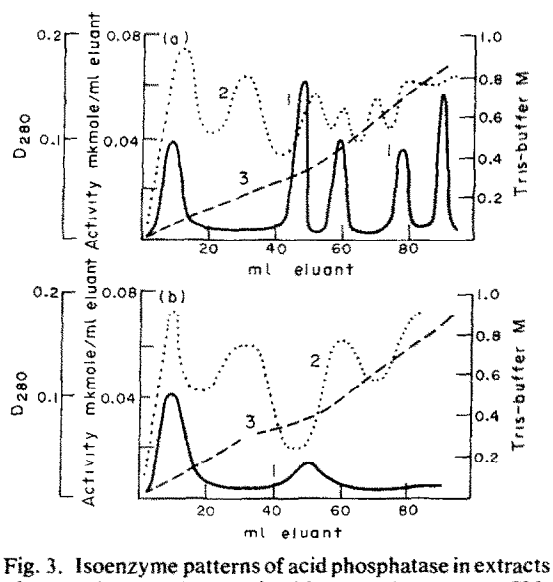

Fig. 3. Isoenzyme patterns of acid phosphatase in extracts of control (a) and treated with N 31 (b) tumors. CM-cellulose column chromotography (see legend to Fig. 2).

enzymes was measured in the presence of 0.1% Triton X-100.

The results are presented as means of three experiments.

Table 1 shows a decrease in acid phosphatase and DNase activity in soluble fractions of treated animals (exceptions were nitrosomethylurea and 5-FU). Free activity of the enzymes isolated from control animals, 35 and 56 per cent of the total for acid phosphatase and DNase respectively. Total activity in test groups is equal to that of control, with the exception of acid phosphatase (in the case of treatment with 5-FU, when a reproducible increase was observed). Free activity in treated cells was equal to total ones. The latter finding indicate a decrease in bound activity and correspondingly, in membrane permeability which is characteristic of increase in efficiency of lysosomes.

Figure 1 compares the effects of thiophosphamide, N31 and N35 as a function of pH. A marked decrease in acid phosphatase activity is observed in extracts after *in vivo* treatment being most pronounced at pH optimum of the enzyme (pH 4.5). The loss in the enzyme activity should be related to reduction in prelysosomal acid phosphatase contents, considering that the activity of extracts is contributed with lysosomal and soluble acid hydrolases, and that total activity reaches free one in treated cells.

The phenomenon was investigated further using chromotographic methods. Extracts of tumor cells after treatment with N31 were used being the most effective of the alkylating agents, and 5-FU. These were subjected to column chromotography on DEAE- or CM-cellulose. The results are presented in Figs 2 and 3. The Figs show that treatment caused a decrease in the number of isoenzymes of acid phosphatase. It was especially pronounced when CM-cellulose was used (two peaks in treated and five peaks in control material).

Thus, activation of lysosomal system has been found in ascite Ehrlich carcinoma cells under *in vivo* administration of antitumor agents, to tumor bearing animals. This effect was accompanied with disappearance of some isoenzymes, isoenzyme patterns becoming sparse.

Previous experiments, *in vitro* with isolated lysosomes or tumor cells showed that antitumor agents of varied classes (and mechanisms of action at concentration span overlapping one available in chemotherapy) do not exert a direct action on lysosomes [9]. Conclusively, lysosome system is not a prime acceptor in antitumor action of drugs. It is probable that the engaging of lysosome system would be secondary and aim at utilization of subcellular structures destroyed by another mechanism, that is at accomplishment of its "scavenger" function.

Laboratory for Experimental Therapy, Petrov Research Institute of Oncology, USSR Ministry of Health, Pesocchy-2, Leningrad, 188646, USSR

V. A. Filov
A. V. Tretyakov
E. M. Ryazanov

REFERENCES

1. A. Allison, *Ad. Chemother.* **3**, 253 (1968).
2. V. A. Filov, *Vopr. Oncol.* **16**, 93 (1970).
3. Z. N. Kapp and S. Okada, *Exp. Cell. Res.* **72**, 473 (1972).
4. C. Todorutiu, D. Daicoviciu, *Rev. roum. Morphol., Embriol., Phisiol. Ser. Morphol., Embriol.*, 21, N3, 219 (1975).
5. S. Abe, S. Sato, *Sci. Rep. Res. Inst. Tohoku Univ.* -C, **21**, 76 (1974).
6. S. Kardaschewics, *Pol. tig. lek.* **27**, 1370 (1972).
7. T. A. J. Grillo, *Afr. J. med. Sci.* **2**, 9 (1974).
8. A. Horvat, O. Touster, *Biochem. Biophys. Acta*, **14**, 725 (1967).
9. V. A. Filov, A. V. Tretyakov, E. M. Ryasanov, *Doklady Acad Nauk* USSR 217, N4, 961 (1974).