INCREASE IN RNase AND DPNase ACTIVITIES IN ASCITES TUMOR CELLS INDUCED BY VARIOUS CYTOSTATIC AGENTS.

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As shown by Green and Bodansky (2), treatment of ascites tumor cells with N-mustard (HN_2) in vivo leads to an increase in DPNase activity which seems to be an induction. A higher capacity to hydrolyze DPN was also observed by Holzer and Kröger (9) in tumor cells treated with the ethylenimine compound E 39 in vivo, although the results were interpreted in a different manner. Experiments performed in our laboratory showed a nearly linear correlation between X-ray induced proliferation inhibition, increase in DPNase activity and the capacity to incorporate nicotinic acid into DPN in ascites tumor cells (8,15). Furthermore, a similar augmentation of RNase activity was observed after in vivo treatment of tumor cells with HN_2 (3), with X-rays (7) and with Trenimon (tris ethyleniminobenzoquinone) (7). In this paper we wish to present similar results using actinomycin, 5-fluorouracil and colchicine as cytostatic agents. In addition, experiments on the response of microsomal and nucleolar DPNase and of free and latent RNase resp. are reported.

METHODS

Ehrlich ascites cell suspensions were drawn from mice 6 days after inoculation with 0.25 ml ascites fluid. Ca 5 ml of the native cell suspension was incubated in Petri dishes with the cytostatic compounds at the given concentrations for 1 hr (25°) or irradiated (c.f. 8) at 0°. 0.25 ml of the control and experimental cell suspensionresp. was injected into mice i.p. (8-10 animals per group)

and the ascites harvested at the 6th day. After detn of total cell volume and cell number of the combined ascites of each group, the cells were centrifuged and lyzed in a 10-20-fold volume of cold water with the aid of all-glass homogenizers. The detn of free RNase activity was made acc. to (16). The detn of free + latent RNase was done by introducing $1x10^{-4}$ M pCMB into the reaction mixture (cf. 14,5). 1 unit causes a change of E_{260} = 1.000 /hr in supernatant under standard conditions (16). DPNase was detnd acc. to Kaplan (10). 1 unit hydrolyses 1 µMol DPN / hr under standard conditions (10). All enzyme detns were run in duplicate.

RESULTS AND DISCUSSION

As summarized in table 1, treatment of tumor cells with a variety of cytostatic agents exhibiting different action mechanisms unvariably leads to cells with increased RNase and DPNase activities resp..

TABLE 1

Inhibition of cell proliferation and increase in free RNase and in DPNase activities per cell after treatment with various cytostatics

Cytostatic Agent (wt/.25 ml ascites)	Cell Prolifer. (increase/mouse)	Free RNase (units/10^6 cells)		DPNase (units/10^8 cells)	
---	$2.91 \pm 1.06x10^8$ = 100 %	1.24 ± 0.45 = 100 %		1.44 ± 0.29 = 100 %	
Actinomycin: 1.6 γ	62 %	3.80	307 %	3.50	243 %
FU: 1.78 mgm	36 %	3.30	266 %	4.80	333 %
3.50 mgm	11 %	4.25	343 %	10.20	708 %
Colchicine: 2.0 γ	13 %	3.95	319 %	2.49	173 %
X-rays: 750 r	26 %	9.98	805 %	-	-
1500 r	25 %	23.00	1855 %	17.22	1195 %
Trenimon: 0.11 γ	46 %	7.05	568 %	13.02	904 %
0.56 γ	2 %	11.40	920 %	22.53	1565 %

Each value represents the mean of the pooled cells of 8-10 mice. Further details see under "Methods".

Similar results could be obtained in different rat tissues with a high proliferation rate (e.g. thymus, spleen) within 6-12 hrs after a single injection of Trenimon (500 γ /kgm), whereas liver showed no response.

The results cannot be explained by a selection of resistant cells having higher enzyme activities since normal growth of tumor cells is resumed after a (dose-dependant) lag period with gradual return to cells exhibiting normal appearance and normal content of enzymes (15). Also, a "host" reaction of the animal can be excluded on the basis of cell culture experiments: Monolayer cultures of HeLa cells respond to a 3 day-treatment with the cytostatic trenimon ($2x10^{-7}$ M) by a 3.5-fold activity of DPNase .

Apparantly, there exists a general correlation between inhibition of cell proliferation and increase in RNase and DPNase activities resp..

Since 5-FU inhibits cell proliferation by inhibiting thymidine and DNA synthesis (cf.13), colchicine by interference with metaphase events(cf. 1), X-irradiation and alkylating agents by a still unknown action on processes intimately connected to cell division (cf.12,15), and actinomycin probably by blockage of the formation of some RNA fraction(s) (cf.11,4), the increase in RNase and DPNase activities resp. seems to be an unspecific re-action of proliferating cells to cytostasis. The elevated enzymatic capacity of proliferation-arrested cells therefore parallels the inhibition of DNA synthesis (15), which can be induced directly (by FU) or indirectly (e.g. by X-rays) [1].

The increase in DPNase activity after X-ray treatment of the cells mainly concerns the microsome-bound enzyme, whereas

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[1] Except for FU and actinomycin resp., none of the cytostatics applied in concentrations to inhibit cell proliferation leads to a primary inhibition of DNA and RNA syntheses resp.. In consequence, giant cells are formed in all instances with a supranormal content of DNA, protein and other cell constituents (cf.12,15,6).

the nuclear[1] DPNase is much less affected (table 2). Since, under the same conditions, an increased capacity of the treated cells to incorporate nicotinic acid into DPN is observed while the

TABLE 2

Preferential increase in microsomal DPNase activity in X-irradiated tumor cells.

X-ray dose	DPNase (units x 10^8 / cell)					
	homogenate		nuclei		microsomes	
---	1.12	100 %	0.46	100 %	0.90	100 %
750 r	4.39	380 %	0.68	148 %	3.34	370 %
1000 r	5.81	520 %	0.54	117 %	5.04	560 %
1500 r	10.95	970 %	1.24	270 %	9.40	1045 %

Typical experiment with 5 animals in each group. Cell proliferation in the control was 1.25×10^8 cells/mouse, after 750 r = 78 %, after 1500 r = 42 % of the control. The combined ascites of each group was centrifuged (10 min at 3000 rpm) and lyzed in a 20-fold volume of 1.8 mM $CaCl_2$ soln using a motor-driven glass homogenizer with teflon pestle until microscopic control revealed <1 % unbroken cells, the nuclei being free of adhering cytoplasm. The nuclei were separated by centrifugation (10 min at 1000 rpm). The microsomes were isolated together with the mitochondria fraction[2] by centrifugation at 105 ooo x g (30 min). The fractions resulting from 1 ml packed cells were taken up in 2.0 ml cold water and homogenized in an all-glass homogenizer. The separation of nuclei from cytoplasmatic constituents in cells with higher irradiation doses is imperfect because of the increased fragility of these nuclei.

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1) The nuclear enzyme is located in the nucleolus and can be clearly distinguished from the microsomal DPNase on the basis of pH optimum, K_i value and type of inhibition by nicotinamide (7).

2) A detailed analysis revealed that, in normal tumor cells, the DPNase activity distribution in twice rewashed fractions is as follows: Nuclei = 24 %, mitochondria = 13%, microsomes = 52 %, and supernatant = 11 %.

level of DPN hardly is affected except after higher doses (>800 r) (8,15), these alterations may be the expression of a stimulated DPN turnover in proliferation-inhibited cells.

The increase in RNase activity, first observed by Green and Bodansky in HN_2-treated tumor cells (3), may also be connected to a stimulated turnover of RNA induced by the inhibition of cell division and of the synthetic processes connected with it. This

TABLE 3

RNase activities in trenimon- and actinomycin-treated tumor cells.

Cytostatic Agent (γ / ml ascites)	Free RNase (units /mgm protein)		Total RNase (units /mgm protein)		Cell Proliferation (cell increase/mouse)	
Trenimon:						
---	0.9	100 %	4.1	100 %	3.08×10^8	100 %
0.4 γ	7.9	878 %	14.2	345 %	1.65×10^8	54 %
2.3 γ	14.0	1550 %	19.2	470 %	0.05×10^8	2 %
Actinomycin:						
---	1.6	100 %	5.1	100 %	2.06×10^8	100 %
1.6 γ	5.2	330 %	10.3	202 %	1.04×10^8	51 %

Separate experiments with 8-10 animals in each group each time. Further details are described under "Methods".

interpretation gains support from the fact that with increasing doses not only total RNase is elevated but also the ratio: free RNase / latent RNase (table 3). Preliminary experiments indicate an augmentation of the cytoplasmatic enzyme(s) mainly.

SUMMARY

Inhibition of ascites tumor cells by a variety of cytostatic agents

having different action mechanisms (X-rays, tris ethylenimino-benzoquinone (Trenimon), colchicine, 5-fluorouracil and actinomycin) leads to cells with elevated RNase and DPNase activities resp.. The increase is correlated with the degree of proliferation inhibition and reaches values >15 times normal.

The elevated DPNase activity mainly concerns the microsomal enzyme, whereas the nuclear (nucleolar) isoenzyme shows but a minor response.

The increase in RNase comprises both free and latent RNase, higher doses leading to a shift much in favor of free RNase.

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