ELEVATION OF DEOXYRIBONUCLEASE ACTIVITIES IN HELA CELLS TREATED WITH SELECTIVE INHIBITORS OF DNA SYNTHESIS

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We have recently reported that treatment of a strain of HeLa cells with mitomycin C (MC) results in an elevation of the activities of cellular deoxyribonucleases (DNases) (Studzinski and Cohen, 1966), and increases in DNase activity have also been found following MC treatment of other types of mammalian cells (Niitani et al., 1964; 1966) and of bacteria (Nakata et al., 1961; Kersten et al., 1965). However, this finding need not be taken to support the view of Kersten (1962, 1964) that MC exerts its inhibitory action on cell division by activating cellular DNases and thus depolymerising cell DNA, because the elevation of DNase activity was not accompanied by a decrease in cell DNA or an increase in acid soluble degradation products of DNA. The results presented in this communication argue further against a role of DNases in the mechanism of action of MC on HeLa cells. It was found that elevation of DNase activities was produced not only by treatment with MC but also by all other specific inhibitors of DNA synthesis that were tested at growth inhibitory concentrations, and that inhibition of HeLa cell proliferation could be produced by MC without significant increase in DNase activity.

Materials and Methods: Culture conditions, enzyme assay methods and analytical procedures have already been described (Studzinski and Ellem, 1966; Studzinski and Cohen, 1966). The only change in the procedure was that the cells were no longer harvested in the culture medium, but by scraping after treatment with a solution of versene. In addition to the strain of HeLa cells previously used, -af of Saksela et al. (1961), HeLa (S3) cells originally obtained from Dr. N. P. Saltzman were also employed in this study. Both strains were free of PPLO contamination. MC, hydroxyurea (HU), nitrogen mustard (HN2) and thymidine (Td R) were obtained from commercial sources. Amethopterin was a gift from Dr. Ing, Cancer Chemotherapy National Service Center, and 5-fluorouracil-2'-deoxyriboside (FUDR) from Dr. W. E. Scott, Hoffmann-La Roche.

Results: The data presented in Table I show that treatment with a number of antimetabolites increased the activities of both DNases studied. The drugs were chosen for their known selective inhibition of DNA synthesis, and were used in concentrations which were sufficient to arrest cell proliferation. Treatment with non-inhibitory concentrations did not lead to increased enzyme activity. HU appeared to be the most effective antimetabolite as regards elevation of DNase activities, but this could perhaps be due to fortuitously suitable combination of drug concentration and length of treatment. After 48 hours of treatment both DNases increased approximately equally with all inhibitors except amethopterin; in this case the increase in acid DNase activity was not seen in some experiments.

The elevation of DNase activity was not accompanied by significant decrease in cell DNA content (Table I). On the contrary, DNA content per cell was significantly higher after treatment with MC, HN2 and Td R. Increases in cell protein content were produced by all the inhibitors (Table I), and this

Table I. DNase activities and cell DNA content of HeLa (-af) cells treated with inhibitors of DNA synthesis.

Inhibitor and concentration in ug per ml	Number of expts.	Cell number per culture $\times 10^{-6}$	Acid DNase		Alkaline DNase		ug DNA per 10^{-6} cells	ug protein per 10^{-6} cells
			Units per 10^6 cells	% of control	Units per 10^6 cells	% of control		
None - 0 time	4	6.13	53(39-59)	-------	30(18-45)	-------	12.8	-------
None - 42 hrs.	8	18.29	69(43-92)	100	33(21-42)	100	12.9	332
Mitomycin C, 0.1	8	6.84	163(101-228)	234	83(55-117)	251	16.8	542
HN2, 2.0	4	5.92	181(112-387)	261	70(49-82)	212	15.1	503
FUDR, 4.4	4	6.52	160(115-213)	231	84(73-102)	254	11.5	564
Amethopterin, 0.5	4	6.30	90(55-136)	131	68(51-75)	208	12.0	428
Hydroxyurea, 60	6	7.03	186(135-236)	267	103(81-119)	313	12.4	550
Thymidine, 242	4	6.73	137(124-191)	197	92(81-101)	279	15.8	471

Each individual experiment was performed in triplicate. The values for enzyme activity are given as mean and range in units defined previously (Studzinski and Cohen, 1966). The inhibitors were allowed to act for 42 hours in each experiment. Calculation of enzyme activities per mg of protein showed that the specific activities were considerably elevated in the treated cells.

increase was taken as an index of "unbalanced growth" (Cohen and Barner, 1954; Rueckert and Mueller, 1960), since in a companion study, cell protein content was found to parallel closely cell RNA content and cell volume (Cohen and Studzinski, 1966).

Table II. Effect of mitomycin C on cell proliferation, cell protein content, and DNase activities of HeLa (S3) cells.

Concentration of MC in ug/ml and duration of treatment	Cell number per culture $\times 10^{-6}$	ug protein per 10^6 cells	Acid DNase units per 10^6 cells	Alkaline DNase units per 10^6 cells
None - 0 time	2.43	389	--------	--------
None - 42 hrs.	7.59	329	111 (55-190)	54 (29-67)
0.05 - 42 hrs.	2.81	372	133 (98-180)	39 (23-57)
0.10 - 42 hrs.	2.03	346	113 (95-145)	58 (51-63)

Mean values of 3 experiments. The range of values for enzyme activity is given in parentheses.

Under certain conditions inhibition of cell multiplication could be produced by MC although no increase in cell DNases was observed. One instance was afforded by treatment with MC of a line of HeLa cells different from the one used in the majority of these experiments — HeLa (S3) cells. Concentrations of MC used were effective in arresting cell proliferation, but the DNase response was variable — in some experiments decreases rather than increases in DNase activities were observed — so that no significant increase in DNase activity was noted in this group of experiments (Table II). It should be noted, however that cell protein content indicated that "unbalanced growth" did not take place in these experiments. Secondly, treatment of HeLa (-af) cells with high concen-

tration of MC, inhibitory to protein as well as DNA synthesis (Lerman and Benyumovich, 1965), rapidly arrested cell division but again DNase activities did not increase (Table III).

Table III. Effect of a high concentration of mitomycin C (50 ug/ml) on DNase activities of HeLa (-af) cells.

Duration of treatment in hours	Acid DNase*		Alkaline DNase*	
	Values based on activity per cell	Values based on specific activity	Values based on activity per cell	Values based on specific activity
2	98	101	109	111
4	102	89	103	98
8	105	125	70	78
16	117	126	27	33
24	68	104	17	26
48	-----	-----	0	0

* The values for enzyme activity are arithmetical means of two similar experiments, expressed as percentages of the control values.

Discussion: The finding that the inhibitors of DNA synthesis with well elucidated and differing mechanisms of action (Alexander and Lett, 1960; Cohen et al., 1958; Iyer and Szybalski, 1963; Morris and Fisher, 1960; Osborn et al., 1958; Young and Hodas, 1964) all produce increases of DNase activities in the treated cells indicates that it is unlikely that MC exerts its inhibitory effect on HeLa cell proliferation through elevation of cellular DNase activity. It would seem rather that the higher cell content of these enzymes is a consequence of "unbalanced growth", however produced, provided only that protein synthesis remains unimpaired.

It would be tempting to speculate that the elevated activity of at least one of the DNases studied here is in some way related to attempted or actual repair of DNA damaged by the inhibitors, since a DNA repair system has been recently shown to be operative in HeLa cells (Rasmussen and Painter, 1966), and repair implies excision of damaged segments of DNA, as was shown for HeLa cell DNA alkylated with sulfur mustard (Crathorn and Roberts, 1966). However, at present there is no evidence to support this hypothesis, and it is equally lidely that in "unbalanced growth" there is increased cell content of various enzymes (cf. Eker, 1966; Erbe et al., 1966; Kit et al., 1966; Niitani et al., 1964, 1966).

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