DEOXYADENOSINE-INDUCED STIMULATION OF THYMIDINE KINASE ACTIVITY OF CHANG CELLS

P. Eker[1]

Norsk Hydro's Institute for Cancer Research, The Norwegian Radium Hospital, Montebello, Oslo, Norway

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We have previously shown that thymidine kinase activity of Chang liver cell cultures is markedly stimulated after treatment with various selective inhibitors of DNA synthesis, such as amethopterin, 5-fluorodeoxyuridine and hydroxyurea (Eker, 1966;Eker, 1968). The effect of these drugs on the kinase level is probably due to induced changes in the intracellular concentration of metabolites which repress enzyme production. Similar observations have been made with other mammalian cells (Stubblefield and Mueller, 1965; Kim et al., 1967).

In the present communication it is shown that treatment of Chang cells with deoxyadenosine at concentrations which strongly inhibit DNA as well as RNA and protein production leads to a similar stimulation of thymidine kinase activity.

METHODS

Cultivation of human liver cells (Chang)[2] and the procedure

1. Fellow of the Norwegian Cancer Society (Landsforeningen mot Kreft).
2. The cells were obtained from Microbiological Associates, Inc., Washington 14, D.C.

used for assay of thymidine kinase activity of cell homogenates have been described previously (Eker, 1965; Eker, 1965). One unit of enzyme is defined as that amount which phosphorylates 1 mμmole of deoxythymidine per minute under standard incubation conditions. Specific activity is expressed as the number of enzyme units per mg of protein. Protein was determined by the Lowry method (Lowry et al., 1951) as modified by Oyama and Eagle (1956). After extraction of cells (Whitfield and Nixon, 1959), DNA was determined by the indole method of Ceriotti (1952) and RNA by the orcinol method (Mejbaum, 1939).

RESULTS AND DISCUSSION

Deoxyadenosine has been reported to be a potent inhibitor of DNA synthesis in Ehrlich ascites tumor cells (Klenow, 1959; Langer and Klenow, 1960; Prusoff, 1959), HeLa cells (Kim et al., 1965; Young and Hodas, 1965) and Chick embryo (Maley and Maley, 1960). In the present study it was found (Table I) that growth of Chang

TABLE I

Effect of Deoxyadenosine[1] on Growth and Production of DNA, RNA and Protein

Concentration of deoxyadenosine (mM)	Cells/flask	Increase per culture (μg)		
		DNA	RNA	Protein
0 (Control)	4.8×10^6	21	78	736
0.5	3.0×10^6	5	29	398

[1] The cells (2.6×10^6 cells/flask) were treated with deoxyadenosine for 20 hours.

liver cells was strongly inhibited by 0.5 mM deoxyadenosine. The total content of DNA, RNA and protein in the treated cultures increased by only about 25%, 40% and 50%, respectively, as compared to untreated cultures. These data strongly indicate that not only DNA synthesis, but also RNA and protein synthesis in the cultures is markedly inhibited by deoxyadenosine. Inhibition of RNA (Kim et al., 1965; Klenow, 1959) and protein (Kim et al., 1965) synthesis by high concentrations of deoxyadenosine (in the range 2-7 mM) has previously been observed in HeLa cells and Ehrlich ascites cells.

From the results shown in Fig. 1 it appears that treatment of liver cell cultures with deoxyadenosine at concentrations which inhibited growth induced a pronounced increase in the activity of thymidine kinase. The mechanism underlying the stimulating effect of deoxyadenosine is not known. It has been shown that

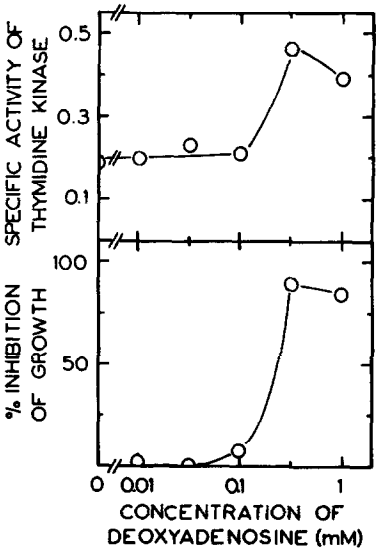

Fig. 1. Effect of deoxyadenosine on growth and thymidine kinase activity. Cell counts and enzyme assay were carried out after incubation for 20 hours. Per cent inhibition of growth was calculated from the observed increases in the total number of cells per culture.

inhibition of DNA synthesis in Ehrlich ascites cells by deoxyadenosine is probably due to the accumulation of dATP in the cell (Munch-Petersen, 1960; Klenow, 1962), and it has been suggested (Klenow, 1962) that this leads to inhibition of the reduction of guanosine and cytidine nucleotides to the corresponding deoxynucleotides. In view of these data it was of interest to learn whether addition of deoxyribonucleosides to the medium would prevent the effects of deoxyadenosine on the liver cells.

TABLE II

Effect of GdR, CdR, TdR and Actinomycin D on Thymidine Kinase Activity of Deoxyadenosine-Treated Cells.

Compounds added	% Inhibition of growth	Specific activity of thymidine kinase
None (Control)	-	0.212
AdR	92	0.509
AdR and GdR	83	0.572
AdR and CdR	92	0.515
AdR and TdR	91	0.554
AdR, GdR, CdR and TdR	86	0.493
AdR and actinomycin D	-	0.192

The medium of exponentially growing cultures (3×10^6 cells/flask) was replaced by fresh medium containing AdR (0.5 mM), GdR (0.05 mM), CdR (0.05 mM), TdR (0.05 mM) and actinomycin D (1 μg/ml) as indicated. Cell counts and enzyme assay were carried out after 20 hours of incubation.

The data in Table II show that addition of deoxyguanosine, deoxycytidine and deoxythymidine neither prevented the stimulation of thymidine kinase activity, nor altered the inhibition of cell growth caused by deoxyadenosine. On the other hand, when actinomycin D was added to the medium simultaneously with deoxyadenosine, the rise in the kinase level did not occur. Although both RNA and protein production in the liver cell cultures appears to be strongly inhibited after 20 hours of treatment with deoxyadenosine (Table I), the results obtained with actinomycin D suggest that the observed rise in thymidine kinase activity may be due to an early and transient increase in protein synthesis.

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