THYMIDINE SENSITIVITY AND DEOXYNUCLEOTIDE POOLS OF HUMAN LYMPHOID
AND MELANOMA CELLS IN VITRO*

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INTRODUCTION

Exposure of cells to excessive amounts of thymidine (dThd) may cause inhibition of DNA synthesis due to a depletion of deoxycytidine 5'-triphosphate (dCTP). Thymidine 5'-triphosphate (dTTP) accumulates in dThd-treated cells and leads to inhibition of ribonucleotide reductase-mediated synthesis of cytosine deoxynucleotides¹. The toxic effects of dThd have been investigated with various mammalian cells in vitro. Notably, lymphoid cells of T-cell origin, but not of B-cell origin, are highly sensitive to dThd^{2,3}. In vitro and in vivo studies have been reported demonstrating the therapeutic potential of high-dose dThd treatment against melanoma⁴⁻⁶. However, no deoxynucleotide metabolism studies on melanoma cells have been reported. In the present study we compared human T- and B- cells and melanoma cells in vitro with respect to dThd sensitivity, deoxynucleotide pool profiles and changes in deoxynucleotide levels in response to dThd.

MATERIALS AND METHODS

Cultivation of lymphoid and melanoma cells was performed as described earlier⁷. Human lymphoblast cell lines, CCRP-CEM (T-cells) and NC37 (B-cells), were grown in stationary suspension culture in RPMI 1640 medium. Human melanoma cell lines, M5 and IGR3, were grown in monolayer in Dulbecco's MEM. Both media contained 15% dialyzed, heat-inactivated fetal bovine serum, and all cell lines were maintained in exponential growth.

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Table 1. Deoxynucleoside Triphosphate Pools in Lymphoid and Melanoma Cells^a

Cell line	dTTP	dCTP (pmol/10 ⁶ cells)	dATP	dGTP
CCRF-CEM	65 ± 26	28 ± 6	66 ± 13	36 ± 9
NC37	72 ± 10	35 ± 6	19 ± 4	4.7 ± 3.0
IGR3	154 ± 38	99 ± 9	96 ± 10	35 ± 13
M5	172 ± 45	63 ± 18	137 ± 31	37 ± 18

^aValues represent mean ± S.D. for 3-6 determination.

Cellular deoxynucleotide pools were determined using high pressure liquid chromatography (HPLC). Flasks with lymphoid cells were cooled for 10 min in ice-water before collection of cells by centrifugation at 800 g for 15 min. Melanoma cells were harvested by rapid trypsinization (2-3 min) followed by suspension of detached cells in cold medium and centrifugation. Extraction of cells and determination of deoxynucleoside triphosphates by HPLC on a strong anion-exchange column is described in detail elsewhere⁸. dTTP, dCTP, deoxyadenosine 5'-triphosphate (dATP) and deoxyguanosine 5'-triphosphate (dGTP) pools were expressed as pmol/10⁶ cells.

RESULTS

Effects of dThd on cell growth was tested with continuous exposure to dThd for 48 hr. dThd sensitivity of the 4 cell lines, which had similar rates of growth (22- to 26-hr doubling times), varied markedly. Concentrations of dThd required for 50% inhibition of growth for CCRF-CEM, IGR3, M5 and NC37 were 12, 120 190 and 1000 μ M, respectively. Inhibition of more than 90% growth of CCRF-CEM, IGR3, M5 and NC37 occurred at dThd concentrations of 0.1, 1, 3 and >10 mM, respectively.

Deoxynucleotide pools were examined in untreated cells and are shown in Table 1. Melanoma cell had higher dTTP and dCTP levels than

Table 2. Deoxynucleoside Triphosphate Pool Profiles of Lymphoid and Melanoma Cells Based on Ratios to dCTP^a

Cell line	dTTP/dCTP	dATP/dCTP	dGTP/dCTP
CCRF-CEM	2.4 ± 1.1	2.5 ± 0.6	1.3 ± 0.4
NC37	2.1 ± 0.5	0.54 ± 0.16	0.14 ± 0.09
IGR3	1.6 ± 0.5	0.96 ± 0.17	0.37 ± 0.17
M5	2.8 ± 0.8	2.2 ± 0.2	0.53 ± 0.22

^aRatios were calculated from deoxynucleotide profiles of 3-6 individual cell samples and expressed as mean ± S.D.

lymphoid cells, while NC37 had the lowest purine deoxynucleotide pools. In terms of ratios of deoxynucleotide to dCTP, dTTP/dCTP was similar for all cell lines, while dATP/dCTP and dGTP/dCTP were quite variable. dGTP/dCTP was highest for CCRF-CEM, lowest for NC37 and intermediate for the melanoma cells (Table 2). Changes in deoxynucleotide pools after 24-hr exposure to dThd at various concentrations for lymphoid (Fig. 1A) and melanoma (Fig. 1B) were examined.

Fig. 1. Deoxynucleoside triphosphate pools in lymphoid (part A) and melanoma cells (part B) after 24-hr exposure to dThd. dTTP, ●; dCTP, ○; dATP, ▲; dGTP, △.

Except in CCRF-CEM cells, 10 μ M dThd produced a less than 2-fold increase in dTTP level with minor changes in other deoxynucleotide pools. At 100 μ M dThd, which had a differential antiproliferative effect on the 4 cell lines, increases in dTTP levels corresponded with sensitivity to dThd, although difference between M5 and NC37 cells was small. However, at this latter dThd concentration, dCTP pool was markedly depleted in CCRF-CEM cells, partially reduced in both melanoma cell lines and unaffected in NC37 cells. At 1 mM dThd, there was a greater increase in dTTP levels in the more sensitive cells as with 100 μ M dThd and there was an attendant, several-fold increase in dGTP pool. However, it should be noted that elevated dGTP levels in NC37 cells were still much lower than in the melanoma cells and there was an only slight decline in dCTP level of NC37 cells as opposed to a marked decline in dCTP level in melanoma cells.

DISCUSSION

A comparison was made between human lymphoid and melanoma cells in vitro regarding growth-inhibitory effect of dThd and deoxynucleotide metabolism. Melanoma cells relative to T- and B-cells had an intermediate sensitivity to dThd. Reported clinical studies on the treatment of leukemias and lymphomas with high-dose dThd have demonstrated limited effectiveness for T-cells and poor results for B-cells^{3,9}. Based on our limited in vitro data, dThd would be expected to yield less than satisfactory results in the treatment of melanoma.

Results of deoxynucleotide studies showed that for the cell lines examined increase in dTTP pool and depletion of dCTP pool correlated, in general, with dThd sensitivity. There have been several studies reported which have investigated various biochemical characteristics in relationship to sensitivity to dThd. With regard to the metabolic processes which may control the accumulation of dTTP, dThd kinase, dThd phosphorylase and 5'-nucleotidase have been assessed as potential determinants of dThd toxicity^{3,10}. We also studied dTTP catabolism in intact cells, whereby cells which were treated with 1 to 10 mM dThd for 3 hr were washed free of dThd, allowed to incubate for an additional 1 hr and examined for changes in dTTP pools. Percent decrease in elevated dTTP levels was greatest for NC37 (87%) followed by that for IGR3 (31%) and for CCRF-CEM (22%). These latter data indicate an appreciable difference for NC37 cells compared to the other cell lines but not between melanoma cells and T-cells. The findings on deoxynucleotide profiles suggest an additional factor which could control dThd activity. The ratio of dGTP to dCTP was found to correlate with sensitivity of cells to dThd. dTTP enhances dGTP levels which in turn promote increases in dATP levels. Purine deoxynucleotides also suppress dCTP synthesis through their inhibitory effects on ribonucleotide reductase and may contribute to the dTTP effect on dCTP. In cells with inherently low levels of purine deoxynucleotides dThd-mediated expansion of these deoxy-

nucleotide pools may not be sufficient to impart inhibitory effects on ribonucleotide reductase activity.

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