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Biochemical and Cell Cycle Perturbations in Methotrexate-Treated Cells

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SUMMARY

The effects of methotrexate (MTX) in the presence or absence of exogenous thymidine (dThd, 10^{-5} M) or hypoxanthine (H_x, 10^{-4} M) on cell cycle kinetics and deoxyribonucleoside triphosphate pools (dNTP) were studied in cultured human leukemic T-cells (CCRF-CEM). MTX cytotoxicity was found to increase linearly with drug dose for MTX concentrations between 10^{-9} M and 10^{-7} M. No further increase in cytotoxicity was observed with much higher MTX concentrations (10^{-7} M–10^{-4} M). A similar dose-response relationship was found for both MTX-induced inhibition of DNA synthesis and changes in dTTP and dGTP pools but not for either MTX-induced inhibition of purine synthesis or changes in dATP and dCTP pools. Exogenous dThd reduced MTX cytotoxicity, at all MTX concentrations examined, but Hx reduced cytotoxicity only at MTX concentrations $<6 \times 10^{-8}$ M and potentiated toxicity with higher MTX concentrations. This potentiation of cytotoxicity was accompanied by substantial elevation of dATP pools. In all instances where dThd or Hx reduced MTX cytotoxicity, a concomitant increase in both dTTP and dGTP levels and in the rate of DNA synthesis was observed. These results suggest a close correlation between MTX-induced alterations of dNTP and inhibition of DNA synthesis and subsequent MTX cytotoxicity. The possible modulation of MTX cytotoxicity by purines is discussed.

INTRODUCTION

The cytotoxicity of MTX[1] and other inhibitors of dihydrofolate reductase is thought to be due to disturbance of *de novo* thymidylate and/or purine biosynthesis. However, the exact mechanism by which MTX treatment kills cells has not been identified, and induction of unbalanced cell growth (1-3), inhibition of purine biosynthesis (4, 5), DNA damage due to misincorporation of deoxyuridine monophosphate (6), and impaired DNA repair caused by perturbed dNTP pools (7) have all been proposed.

We have recently reported that the cytotoxic effects of MTX in a cultured human leukemic T-cell line (CCRF-CEM) appeared to be closely related to inhibition of DNA synthesis and that the occurrence of unbalanced cell growth was unrelated to MTX cytotoxicity (8). In the present study we report changes in cell kinetic parameters and in dNTP levels of CCRF-CEM cells exposed to MTX in the presence and absence of exogenous Hx and/or dThd. Most previous studies of dNTP levels in MTX-treated cells have reported a reduction in dTTP concentration, but the effects on the other dNTP have been variable (9-15). Our results indicate that the effects of MTX and nucleosides on dNTP levels and cytotoxicity are both concentration- and time-dependent. These findings might in part explain the variability of MTX effects on dNTP pools reported by other studies which generally have been confined to single MTX concentrations and exposure times.

[1] The abbreviations used are: MTX, methotrexate; dNTP, deoxyribonucleoside triphosphate; Hx, hypoxanthine; dThd, thymidine; RPMI-1640; Roswell Park Memorial Institute medium, Type 1640.

MATERIALS AND METHODS

Chemicals. dThd and Hx were obtained from Calbiochem (Australia) Pty Ltd. MTX was a gift from Dr A. Hellestrand, Cyanamid (Australia) Pty Ltd. Stock solutions of dThd and MTX were made up at 30 times final concentration in RPMI-1640 medium without fetal calf serum. Hx was dissolved in 10 mM sodium hydroxide solution, heated at 60° for 30 min, then diluted to 30 times the required final concentration with RPMI-1640 medium. Stock solutions were sterilized by Millipore filtration and stored at −20° until required.

^{3}H-Labeled deoxyribonucleoside triphosphates were purchased from the Radiochemical Centre (Amersham, United Kingdom). Unlabeled deoxyribonucleoside triphosphates were purchased from Sigma Chemical Company (St. Louis, Mo.) and P-L Biochemicals (Milwaukee, Wisc.). *Micrococcus luteus* DNA polymerase and templates for the DNA polymerase assay for deoxyribonucleoside triphosphates, poly d(A-T) and poly d(I-C), were purchased from Miles Laboratories (Elkhart, Ind.).

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Cell culture. Human leukemic T-cells (CCRF-CEM) (16) were grown as a suspension culture at 37° in RPMI-1640 medium supplemented with L-glutamine and 10% fetal calf serum. The cell population doubling time was about 22 hr. In all experiments, 75 cm^2 tissue culture flasks (Corning Glass Company, Corning, N. Y.) were inoculated with 90–150 ml of cell suspension at 10^5 cells/ml. The cultures were then allowed to grow undisturbed for 24 hr before addition of drugs. All treatments were carried out on exponentially growing cultures. Cell counts were made by phase-contrast microscopy, which allows discrimination between live (phase-positive) and dead (phase-negative) cells. All cell counts shown are of viable cells only.

In all experiments the drugs were added as a small volume directly to cells in suspension, 24 hr after culture initiation. Each drug was administered as a single dose and remained in the culture for the duration of the experiment. When drug combinations were tested the drugs were added simultaneously. An appropriate volume of the drug vehicle was added to control flasks.

Flow cytometry. Measurements of cellular DNA content and correlated two-parameter analysis of DNA and RNA were carried out using an ICP 22 flow cytometer (Ortho Instruments, Westwood, Mass.). Cells were stained for DNA content using ethidium bromide/mithramycin. The metachromatic dye acridine orange was used for two parameter analysis of DNA and RNA. Acridine orange intercalates with double-stranded nucleic acids and dye-stacks on single-stranded nucleic acids which, when excited with blue light, will cause green and red fluorescence, respectively. The staining conditions used are such that green fluorescence provides a measure of cellular DNA content and the red fluorescence, cellular RNA content (17). With the ICP 22 flow cytometer in coincident mode, this staining technique allows the simultaneous measurement of both DNA and RNA within individual cells. The data accumulated in this fashion from a population of cells may then be displayed as a frequency distribution contour plot. We have previously shown that increases in red fluorescence with MTX treatment result from increases in cellular RNA content (8). Detailed descriptions of staining procedures for DNA and for DNA and RNA have been previously described (8, 18).

DNA polymerase assay for dNTP. This assay was performed as previously described by us (12), except that the cells were extracted with 60% ethanol rather than 60% methanol. This assay is a modification of the method described by Solter and Handschumacher (19).

Measurement of ATP. Assays for ATP were carried out using purified firefly luciferase in a bioluminescence technique previously described by Thore (20). The reagents required for the assay were supplied in kit form by LKB-Wallac (Turku, Finland).

RESULTS

Effects of MTX on cell growth. The effects of MTX on the growth of CCRF-CEM cells over a period of 72 hr is shown in Fig. 1. With MTX concentrations between 10^{-9} M and 10^{-7} M, a concentration-dependent inhibition of cell growth was observed, but concentrations of 10^{-7} M–10^{-4} M had no further effect on cell growth. An increasing inhibition of cell cycle progression, shown by the accumulation of cells with an S-phase DNA content, was also observed at 24 hr for MTX concentrations up to 2×10^{-8} M (Fig. 2). However, at MTX concentrations of 6×10^{-8} M or greater, only minor changes were seen in the DNA content distributions when compared with controls. Our previous studies (8) have suggested that this latter effect may be due to the complete inhibition of cell cycle progression through both G_1 and S phases.

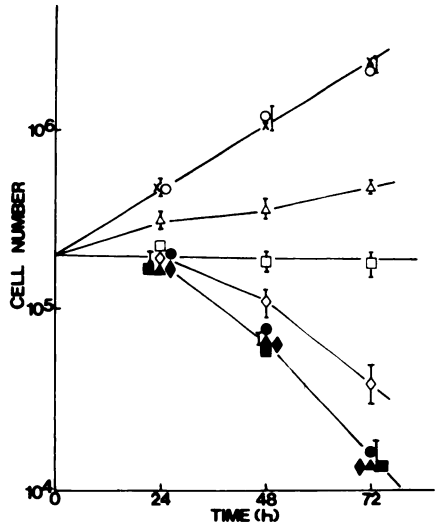

FIG. 1. *Effects of MTX on the growth of CCRF-CEM cells in culture* ×, Untreated controls; ○, 10^{-9} M; △, 10^{-8} M; □, 2×10^{-8} M; ◇, 6×10^{-8} M; ●, 10^{-7} M; ▲, 10^{-6} M; ■, 10^{-5} M; ◆, 10^{-4} M. Error bars represent ± 2 SE from the mean obtained from four individual experiments. Counts are of live cells only as measured by phase-contrast microscopy.

Correlated two-parameter analysis of acridine orange-stained cells was used to study the relative effects of MTX treatment on DNA and RNA synthesis. The relationship between RNA content (red fluorescence) and DNA content (green fluorescence) for control cells and the effects of MTX treatment is shown in Fig. 3. It can be seen from the increases in red fluorescence that MTX concentrations between 2×10^{-8} M and 10^{-6} M resulted in classical unbalanced growth (i.e., an excessive RNA content compared with the corresponding DNA content). No unbalanced cell growth was seen with either 10^{-8} M or 10^{-4} M MTX, although perturbations of DNA synthesis were observed in both instances.

The effects of exogenous dThd (10^{-5} M) and/or Hx (10^{-4} M) on MTX-treated cells (10^{-8} M, 2×10^{-8} M, 10^{-4} M) were also examined. dThd reduced MTX cytotoxicity at all dose levels of MTX (Table 1) and in each instance this was accompanied by a decrease in the inhibition of DNA synthesis (Fig. 2). With 10^{-8} M MTX, the decrease in inhibition of DNA synthesis with the addition of dThd is shown by the reduction of cells accumulated in S-phase and the increase in numbers of viable cells compared with 10^{-8} M MTX alone. At the higher MTX concentrations this effect is shown by an increase in relative DNA content; i.e., cells have progressed to later stages of the cell cycle compared with those treated with MTX alone.

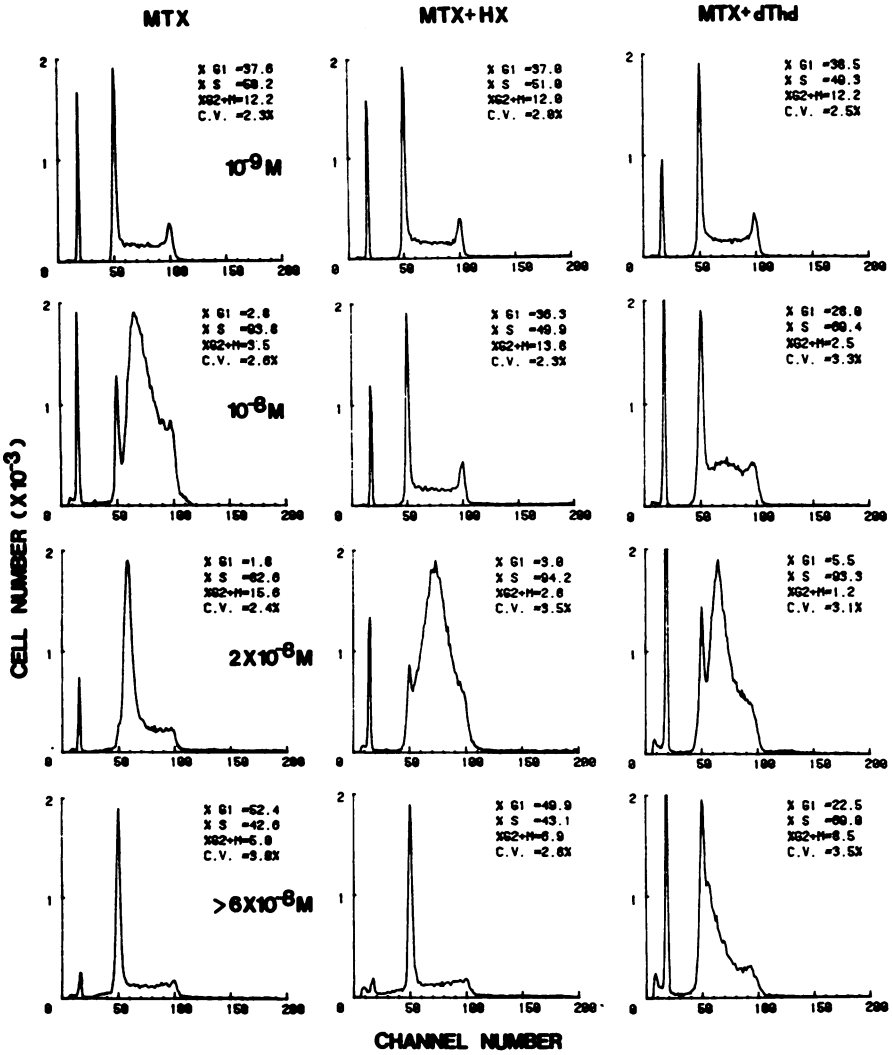

FIG. 2. *Changes in the DNA distribution of CCRF-CEM cells treated with MTX for 24 hr in the presence or absence of dThd (10^{-5} M) or Hx (10^{-4} M)*

The DNA distribution of untreated CCRF-CEM cells (not shown) was *identical* with that found for 10^{-9} M MTX. *Channel number* represents relative fluorescence intensity, which is directly proportional to cellular DNA content. Numbers of cells are shown on the ordinate. The peak between channels *10* and *20* represents chicken red blood cells, which act as an internal biological standard and thus allow correct placement of the G_1 DNA peak which appears in channel *50*. The ratio of the G_1 DNA peak channel number to that of untreated controls was 3.3. The DNA peak of cells treated with 2×10^{-8} M MTX represents cells accumulated in early S-phase as judged from the peak/chicken red blood cell ratio of 3.8. *C.V.*, coefficient of variation of G_1 DNA peak.

At the two lower MTX concentrations, Hx also reduced cytotoxicity (Table 1) and inhibition of DNA synthesis (Fig. 2) to an extent similar to that caused by dThd. However, Hx significantly potentiated 10^{-4} M MTX cell killing and had no "rescuing" effect on DNA synthesis. We have subsequently found that Hx potentiation of MTX cytotoxicity in CCRF-CEM cells occurs with all MTX concentrations in excess of 6×10^{-8} M (data not shown).

The combination of dThd plus Hx itself caused perturbations in cell growth, although no such disturbance with either agent alone was noted (Table 1). The combination completely prevented MTX cytotoxicity at all MTX concentrations studied. However, with 10^{-4} M MTX this "rescue" failed between 48 and 72 hr, as shown by the decrease in numbers of viable cells unless further dThd was added at 48 hr (Table 2).

Effect of methotrexate on dNTP levels. Changes in intracellular dNTP levels of CCRF-CEM cells, 24 hr or 48 hr after exposure to a range of MTX concentrations (10^{-9} M–10^{-4} M), are shown in Fig. 4. At 24 hr 10^{-9} M MTX caused small increases (10%) in both dTTP and dGTP but had no effect on dATP or dCTP. However, with MTX concentrations between 10^{-9} M and 2×10^{-8} M, a linear decline in dATP, dTTP, and dGTP pools to 10–15% of control levels was observed. No further changes in dTTP or dGTP levels were seen with higher MTX concentrations, but dATP levels rose with increasing MTX dose to reach 70% of control levels with 10^{-4} M MTX. A similar dose-response pattern was found for these dNTP pools at 48 hr, although the levels were 2- to 3-fold higher than those at 24 hr, and this resulted in greater than control dATP levels in cells exposed for 48 hr to MTX concentrations between 6×10^{-8} M and 10^{-4}

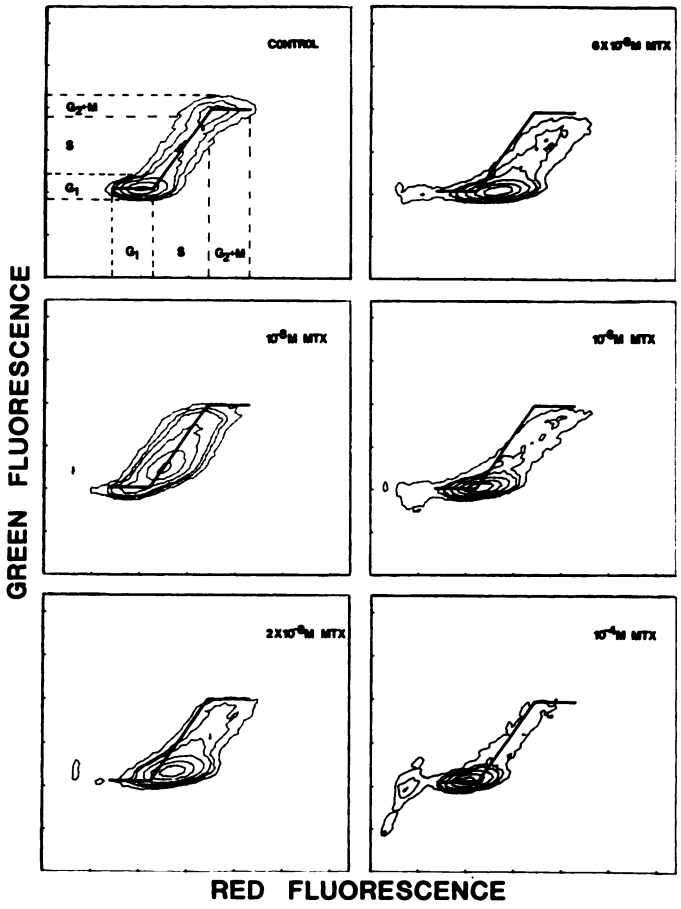

FIG. 3. *Correlated two-parameter analysis of acridine orange-stained CCRF-CEM cells*

Red and green fluorescence represents RNA and DNA, respectively. Each contour plot was generated by using six contour levels of 50, 100, 150, 300, 600, and 1000 cells. To facilitate comparisons between individual plots, a *heavy solid line*, representative of control values, has been superimposed on each contour plot. The drug exposure time in each case was 24 hr.

M. Changes in the dCTP pool with MTX treatment were more complex than those seen in the other dNTP pools. Exposure for 24 hr to MTX concentrations of 10^{-8} M and 2×10^{-8} M caused an elevation of dCTP pool levels to

TABLE 1

Modification of the growth inhibitory effects of MTX in CCRF-CEM cells by dThd (10^{-5} M) and/or Hx (10^{-4} M)

All values represent the mean of four individual experiments ± 2 SE. Counts are of live cells, as judged from phase-contrast microscopy, at 72 hr after treatment. Drug treatment commenced 24 hr after initial seeding of culture flasks with 10^5 cells/ml. Cell cycle time is approximately 22 hr.

MTX	Cell count			
	Control	+dThd	+Hx	+dThd+Hx
M		*×10⁵/ml*		
0	24.0 ± 2.0	23.0 ± 2.8	22.5 ± 3.1	10.0 ± 1.5
10^{-8}	4.9 ± 0.5	11.5 ± 1.0	20.3 ± 2.7	9.8 ± 1.3
2×10^{-8}	1.8 ± 0.4	3.5 ± 0.4	4.5 ± 0.5	9.8 ± 0.3
10^{-4}	0.15 ± 0.04	1.5 ± 0.2	0.05 ± 0.02	2.5 ± 0.5
				10.0 ± 1.1[a]

[a] Additional dThd (10^{-5}M) at 48 hr.

TABLE 2

Changes in dNTP in CCRF-CEM cells with time of exposure to 10^{-4} M MTX plus dThd (10^{-5} M) plus Hx (10^{-4} M)

All values represent the mean of four individual experiments ± 2 SE. Culture conditions are described under Materials and Methods.

Time	Cell no.	dNTP			
		dATP	dCTP	dTTP	dGTP
hr	*× 10⁻⁵*		*% control*		
24	3.7 ± 0.5	166 ± 12	63 ± 2	210 ± 20	165 ± 17
48	6.5 ± 0.7	193 ± 20	80 ± 5	88 ± 6	120 ± 9
72	2.5 ± 0.5	485 ± 53	330 ± 10	21 ± 6	10 ± 1
72[a]	10.0 ± 1.1	199 ± 21	118 ± 4	128 ± 3	150 ± 10

[a] Additional 10^{-5} M thymidine at 48 hr.

greater than control values, but higher MTX concentrations resulted in a dose-independent reduction of dCTP to 70% of controls. As with the other dNTP pools, increasing the duration of MTX exposure to 48 hr resulted in a further increase in dCTP pools compared with those seen at 24 hr. In this case, however, all MTX concentrations between 10^{-8} M and 10^{-4} M resulted in levels of dCTP greater than control levels.

Effects of exogenous dThd and/or Hx on MTX-induced dNTP changes. The effects of exogenous dThd and/or Hx on the MTX-induced changes in dNTP pools are shown in Fig. 5. A concentration of 10^{-5} M dThd alone caused an increase in both dTTP and dGTP, a slight reduction in dCTP, and had no significant effect on dATP levels. The addition of dThd partly prevented the MTX-induced falls in dTTP, and dGTP, and dATP.

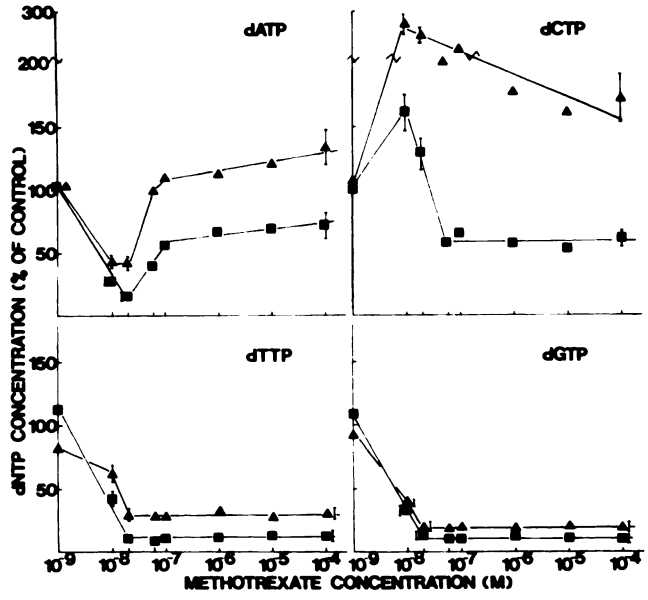

FIG. 4. *Changes in dNTP with MTX treatment*

■, 24-hr drug exposure; ▲, 48-hr drug exposure. The values shown for 10^{-8}, 2×10^{-8}, and 10^{-4} M MTX represent the mean of nine individual experiments ± standard error. All other values represent the mean of three individual experiments. The over-all mean concentrations of dNTP (picomoles per 10^6 cells ± standard error) in untreated cultures of CCRF-CEM cells in the above experiments and for those shown in Fig. 5 were as follows: dATP, 49 ± 6; dTTP, 37 ± 3; dCTP, 23 ± 3; and dGTP, 25 ± 2. No significant difference was observed between measurements of dNTP in untreated cells at 24 hr or 48 hr.

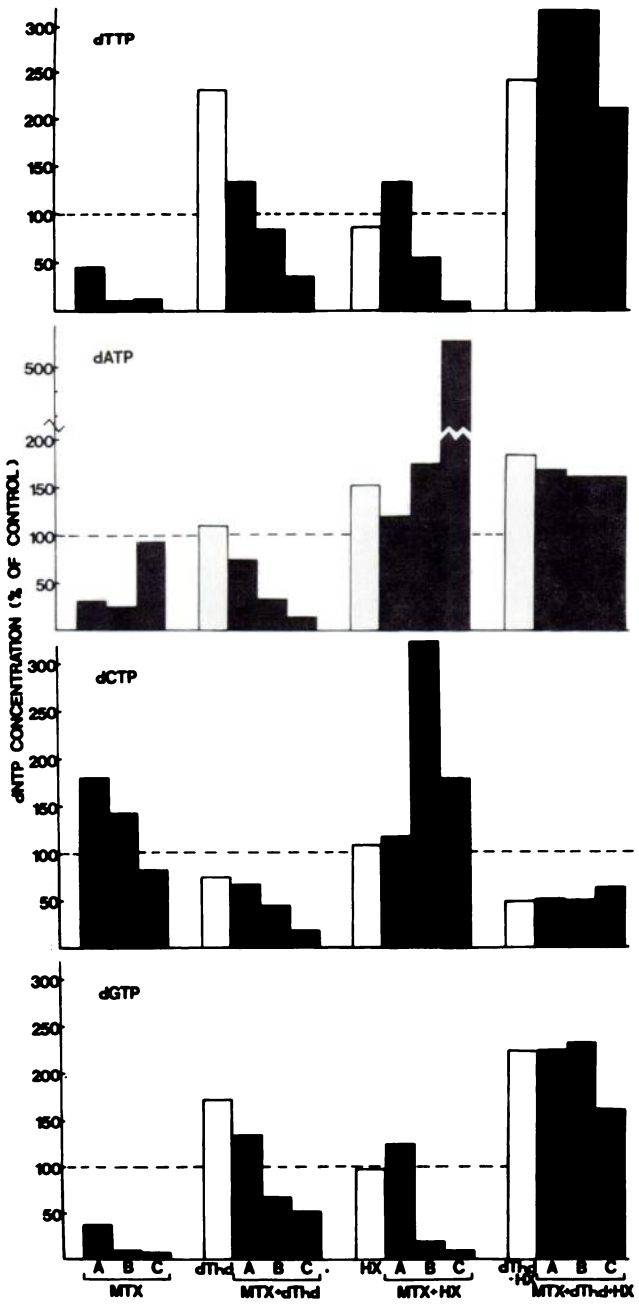

FIG. 5. *Effects of dThd (10^{-5} M) and/or Hx (10^{-4} M) on MTX-induced changes in dNTP in CCRF-CEM cells*

The *open bars* show the effects of dThd and/or Hx in the absence of MTX. The *closed bars* show the effects of several concentrations of MTX in the presence or absence of dThd and/or Hx. *A*, 10^{-8} M MTX; *B*, 2×10^{-8} M MTX; *C*, 10^{-4} M MTX. All measurements were made after 24-hr exposure to drug. The results shown represent the mean of six individual experiments. Scatter ≯ 15% of the mean.

However, the tendency of dThd to reverse partially these MTX-induced pool changes decreased with increasing MTX concentration. The combination of dThd and MTX also resulted in dCTP levels lower than those observed with either MTX or dThd alone.

A concentration of 10^{-4} M Hx caused a 50% elevation in dATP levels and a small decrease in dTTP but had no significant effect on dGTP or dCTP pools. In contrast to dThd, exogenous Hx completely reversed the dNTP changes seen with 10^{-8} M MTX. Hx also completely prevented both the cell cycle perturbations and growth inhibitory effects of 10^{-8} M MTX (Fig. 2 and Table 1). However, with higher MTX concentrations, Hx prevention of the fall in dTTP and dGTP pools progressively decreased, and with 10^{-4} M MTX exogenous Hx had no effect on dTTP or dGTP levels. On the other hand, marked elevations of dATP to 5 times control levels were observed with increasing doses of MTX in the presence of Hx. dCTP levels were also elevated to a lesser extent.

The combination of dThd and Hx in the absence of MTX resulted in elevated levels of dTTP, dATP, and dGTP and a reduction in the dCTP pool, and had a moderate growth inhibitory effect (Table 1). MTX at any of the dose levels did not significantly alter pool changes caused by dThd plus Hx alone. However, dramatic changes in dNTP levels were observed with the combination of 10^{-4} M MTX plus dThd plus Hx when rescue failed after 48 hr; the dATP and dCTP pools increased with concomitant reductions in dTTP and dGTP. These dNTP pool changes paralleled closely those observed at 24 hr for 10^{-4} M MTX plus Hx and could be prevented by the addition of further dThd at 48 hr (Table 2).

Effect of MTX on ATP levels. MTX caused a decrease in ATP pools after 24 hr (Table 3), but this was not linearly related to MTX concentration, and the lowest ATP levels were found with 2×10^{-8} M MTX (as were MTX-induced changes in dATP pools). Hx completely prevented these changes, and elevated ATP pool levels to 2–3 times control levels. In contrast, dThd caused a marked fall in ATP, particularly with higher MTX concentrations, to levels much lower than those seen with MTX alone.

DISCUSSION

The effects of MTX on cell growth are typical of a cell-cycle phase-specific cytotoxic agent in that cytotoxicity increases with increasing drug dose until a plateau is reached when no further cell killing is observed, even with very much greater drug concentrations. The inhibition of cell cycle progression caused by MTX, as measured by flow cytometry, closely parallels the drug's cytotoxic effects. MTX-induced inhibition of DNA synthesis appears to be due to the inhibitory effects of MTX on both thymidylate and purine biosynthesis (8). The relative effects of MTX on DNA and RNA synthesis can readily be determined from the two-parameter flow cytometric analysis of DNA and RNA. A concentration of

TABLE 3

Changes in ATP levels in CCRF-CEM cells with MTX treatment in the presence or absence of dThd (10^{-5} M) or Hx (10^{-4} M)

All values represent the mean of two individual experiments. Measurements of ATP were made at 24 hr after drug treatment. The ATP level of untreated CCRF-CEM cells was 2.5 nmoles/10^6 cells.

MTX	ATP		
	—	+dThd	+Hx
M		*% control*	
10^{-8}	72	85	166
2×10^{-8}	58	33	230
10^{-4}	72	14	270

10^{-8} M MTX partially inhibits DNA synthesis, but no DNA/RNA unbalance occurs, indicating that RNA synthesis is inhibited to a similar extent. With increased MTX concentration, cell exhibit classical unbalanced morphology, showing that the inhibition of DNA synthesis has become more pronounced than that of RNA synthesis. Only at 10^{-4} M MTX is RNA synthesis (and by inference purine synthesis) completely inhibited as demonstrated by the lack of unbalanced growth when DNA synthesis is completely inhibited. These results suggest that inhibition of RNA synthesis per se does not correlate with MTX cytotoxicity.

The extent to which thymidine and/or purines prevent MTX toxicity has been reported to differ among cell lines (1, 2, 4, 14, 21, 22). In the CCRF-CEM cells, both dThd and Hx reduce the toxicity of low doses of MTX, with Hx being the more effective with 10^{-8} M MTX. However, whereas dThd reduced toxicity with all MTX concentrations studied, Hx actually potentiated toxicity with MTX concentrations greater than 6×10^{-8} M. The combination of dThd and Hx completely prevented MTX cytotoxicity at all concentrations of MTX. In all instances where a reduction of MTX cytotoxicity was observed, an increase in DNA synthesis was found. These results and those with MTX alone suggest that drug-induced inhibition of DNA synthesis and cytotoxicity are closely related.

A wide range of effects of MTX on dNTP pools have been reported (9–15), but generally MTX decreases both dTTP and dGTP. The decrease in dGTP pools has been thought to reflect the antipurine effects of MTX (11, 23), and previously reported findings with 5-fluorouracil (12, 13), which decreased dTTP but increased dGTP, tend to support this suggestion. However, in this study we note that changes in dGTP pools mirror changes in dTTP and that Hx does not elevate dGTP pools in the presence of 10^{-4} M MTX. Furthermore, treatment of CCRF-CEM cells with 3×10^{-7} M fluorodeoxyuridine reduced both dTTP and dGTP pools to about 10% of their control levels.[2] Taken together, these data suggest that changes in dTTP, a positive and negative effector for GDP reduction by ribonucleoside reductase (24, 25), may be responsible for changes in dGTP pools. The reduction of dTTP and dGTP pools caused by MTX correlated closely with MTX cytotoxicity in this study.

Increased dCTP pools seen with low doses of MTX at 24 hr have previously been described (11, 13, 14), and this may reflect cell cycle perturbations and accumulation of cells in early S-phase, since dCTP rises disproportionately in early S-phase (26). The decreased dCTP pools at 24 hr caused by higher MTX concentrations may reflect activation of deoxycytidylate deaminase by the low dTTP pools (27).

The dATP pool changes caused by MTX result from alterations in rates of dATP utilization and synthesis. Hx prevented the fall in dATP seen with low MTX concentrations, suggesting that reduced dATP was due to continued dATP utilization and a partial inhibition of purine biosynthesis. On the other hand, the prevention by dThd of the increased dATP pools seen with higher MTX concentrations suggests that reduced dATP utilization was caused by low dTTP pools while some purine biosynthesis continues. This hypothesis is supported by the MTX dose-dependent increases in dATP observed with the combination of MTX and Hx treatment.

Changes in dNTP varied with MTX exposure time. Similar patterns were found at both 8 hr (data not shown) and 24 hr, although the former were generally less marked. dTTP and dGTP showed similar dose-response patterns at both 24 hr and 48 hr, but dATP and dCTP changes differed, presumably reflecting complex cell cycle and metabolic changes in the surviving cells.

The addition of dThd to 10^{-8} M MTX restored both dTTP and dGTP, but not dATP and dCTP, to normal levels. Surprisingly, the effects of dThd on dNTP pools in MTX-treated cells decreased with increasing dose of MTX. Although this might result from impairment of dThd transport caused by MTX, the low ATP levels found with the combination of MTX plus dThd suggest that reduction in thymidine phosphorylation may contribute. The decrease in ATP levels with increasing MTX concentrations in the presence of dThd might be due to continued purine utilization with increasingly impaired biosynthesis in a manner similar to that proposed earlier for dATP fluctuations. Thus, in the presence of excess dThd, MTX exerts a predominately antipurine effect (14).

In contrast to the dThd results, 10^{-8} M MTX and Hx restored all dNTP pools to control levels and completely prevented the effects of MTX on cell growth and cell cycle progression. Hx elevated the dTTP pool in the presence of 10^{-8} M and 2×10^{-8} M MTX, and reduced the inhibition of growth and DNA synthesis. The increased dTTP levels with Hx treatment in the presence of MTX may result from a sparing effect on reduced folate utilization for purine synthesis when dihydrofolate reductase is only partially inhibited. With much higher MTX concentrations (10^{-4} M), where complete inhibition of dihydrofolate reductase might be expected, Hx did not increase dTTP pools and potentiated rather than prevented MTX cytotoxicity. This potentiation of MTX toxicity was accompanied by a substantial increase in dATP levels, and similar increases in dATP levels and cytotoxicity were also found when "rescue" from 10^{-4} M MTX by dThd plus Hx failed at 72 h (due to a deficiency of dThd). Whether the elevation in dATP pools per se is directly responsible for this potentiation of MTX toxicity, perhaps by inhibition of DNA ligase (28), has not as yet been determined. However, MTX concentration-dependent protection/potentiation by Hx also occurs in other cell types.[3]

This study shows that there is close correlation between MTX-induced dNTP pool changes (in particular dTTP and dGTP) and effects on DNA synthesis and subsequent MTX cytotoxicity. The modulating effect of purines on MTX cytotoxicity has been noted, and further investigations are in progress.

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