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MODULATION OF DEOXYNUCLEOTIDE METABOLISM BY THE DEOXYCYTIDYLATE DEAMINASE INHIBITOR 3,4,5,6-TETRAHYDRODEOXYURIDINE*

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Abstract—Tetrahydrodeoxyuridine (dTHU) inhibits deoxycytidine deaminase and, after intracellular phosphorylation to the active 5′-monophosphate, also inhibits deoxycytidylate deaminase (dCMPD). Because *in vitro* studies have shown that dCMPD may regulate pyrimidine deoxynucleotide metabolism, the objective of this study was to investigate the effects of dTHU on deoxynucleotide metabolism in whole cells. Nearly complete inhibition of dCMPD, measured in intact CCRF-CEM cells by incorporation of [^{14}C]dCyd into dTTP, occurred after a 45-min incubation with 100 μM dTHU. This was accompanied by an 8-fold dCTP pool expansion, although dATP, dTTP, dGTP, and ribonucleoside triphosphate pools were unaffected. Tetrahydrouridine, which inhibits deoxycytidine deaminase exclusively, had no effect on nucleotide pools. The dCTP pool expansion was directly proportional to the dTHU concentration (3–100 μM) and reached a maximum after 2 hr. Inhibition of ribonucleotide reductase by hydroxyurea completely prevented the dTHU-induced dCTP pool expansion, indicating that the substrate of dCMPD was derived from the ribonucleotide pool and that CDP was the predominant precursor of dCTP. dTHU-mediated inhibition of dCMPD appeared reversible. Exposure of cells to 100 μM dTHU followed by washing into fresh medium resulted in a linear decrease of the dCTP pool and an increase in the dTTP pool. The increased dCTP concentration after preincubation with dTHU was associated with an inhibition of deoxycytidine kinase, as indicated by a reduced capacity of cells to phosphorylate ara-C. dTHU is a useful new tool for investigating the role of dCMPD in the regulation of deoxynucleotide metabolism in whole cells.

3,4,5,6-Tetrahydrouridine (THU)§ and 3,4,5-6-tetrahydro-2′-deoxyuridine (dTHU) are potent competitive inhibitors of deoxycytidine deaminase (CD) [1–6]. dTHU is phosphorylated intracellularly to the 5′-monophosphate (dTHUMP) and as such inhibits dCMP deaminase (dCMPD) [5, 6]. THU resembles a transition form in the process of Cyd deamination where the C4 carbon of the pyrimidine assumes a tetrahedral position [3]. It has therefore been suggested that THU inhibits CD through its action as a "transition state analog" [3, 7]. A similar mechanism of action may be assumed for the inhibition of dCMPD by dTHUMP. Although several compounds have been demonstrated to inhibit dCMPD, only dTHU shows sufficient potency and specificity to be used as an analytical tool in the investigation of dCMPD function [5].

dCMPD is a key enzyme that connects the biosynthesis of dCTP and dTTP at the level of the dCMP pool [8, 9]. dCMP is synthesized by the "salvage pathway" via dCyd kinase, a reaction that is tightly regulated by the end metabolite, dCTP [10, 11]. The dCMP pool is also fed by the "*de novo* synthesis" pathway mediated by ribonucleotide reductase (EC 1.17.4.1), which converts CDP to dCDP. This pathway is regulated by dTTP [12, 13].

The activity of dCMPD appears to balance the distribution of the dCMP pool between the dCTP and dTTP pools. First, dCMP may be channeled into the dCTP pool via the action of nucleotide kinases [14, 15]. Second, dCMP is deaminated by dCMPD to dUMP, a substrate for thymidylate synthase (TS) in the synthesis of dTMP, which subsequently is phosphorylated to dTTP. Significantly, *in vitro* investigations have demonstrated that the activity of dCMPD is controlled allosterically by the ratio of the activator dCTP and the inhibitor dTTP [16, 17].

The role of dCMPD in the deoxynucleotide metabolism has, in part, been elucidated by the comparison of dCMPD-deficient cell lines with their parent cell lines [9, 18–20]. An expansion of the dCTP pool was observed in several dCMP-deficient cell lines [9, 18–20]. However, it remained unclear whether other enzymatic pathways had been concurrently altered in these dCMPD-deficient variants and had thus

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§ Abbreviations: ara-C, ara-CMP, and ara-CTP, 1-β-D-arabinofuranosylcytosine, its 5′-mono-, and 5′-triphosphate; CD, deoxycytidine deaminase (EC 3.5.4.14); dCyd kinase, deoxycytidine kinase (EC 2.7.1.74); dCMPD, deoxycytidylate deaminase (EC 3.5.4.12); dNTP, deoxynucleoside 5′-triphosphate; dTHU, 3,4,5,6-tetrahydro-2′-deoxyuridine; dTHUMP, the 5′-monophosphate of dTHU; NTP, nucleoside 5′-triphosphate; PBS, phosphate-buffered saline (8.1 g NaCl, 0.22 g KCl, 1.14 g Na_2HPO_4, and 0.27 g KH_2PO_4 per liter of H_2O, pH 7.4); THU, 3,4,5,6-tetrahydrouridine; and TS, thymidylate synthase (EC 2.1.1.45).

contributed to the dCTP pool expansion [9]. In the parent study, we investigated dTHU as a tool in the kinetic analysis of dCMPD-dependent deoxynucleotide (dNTP) modulation. More specifically, this study focused on the relationship between dCMPD activity and dCTP pool metabolism.

MATERIALS AND METHODS

Materials. dTHU was obtained from Behring Diagnostics (La Jolla, CA). THU was provided by Dr. Ven Narayanan, Drug Synthesis and Chemistry Branch, National Cancer Institute. Deoxycytidine, ara-C, ara-CTP, and all natural NTPs were purchased from the Sigma Chemical Co. (St Louis, MO). [2-^{14}C]dCyd (sp. act. 25.2 mCi/mmol) was a product of the New England Nuclear Corp. (Boston, MA). [*Methyl*-^{3}H]Thymidine (sp. act. 50 Ci/mmol) was purchased from ICN Radiochemicals, Inc. (Irvine, CA). Moravek Biochemicals, Inc. (Brea, CA) was the source of [5,6-^{3}H]ara-C (sp. act. 20 Ci/mmol).

Cell line. The T-lymphoblast cell line CCRF-CEM was obtained from the American Type Culture Collection (Rockville, MD). Cells were maintained in suspension culture in RPMI 1640 medium (GIBCO Laboratories, Grand Island, NY) supplemented with 10% heat-inactivated fetal bovine serum (GIBCO) at 37° in a humidified atmosphere containing 5% CO_2. In indicated experiments, fetal bovine serum was dialyzed extensively against phosphate-buffered saline (PBS) to remove endogenous nucleosides. Cell cultures consistently tested negative for mycoplasma contamination (American Type Culture Collection). All experiments were performed with suspension culture cells in exponential growth phase. Cell number and volume were determined by a Coulter counter equipped with a model C-1000 particle size analyzer (Coulter Electronics, Hialeah, FL). The mean cell volume was 9.43×10^{-11} l/cell.

Nucleotide extraction and analysis. Cells were washed with ice-cold PBS and collected by centrifugation; then the pellet was extracted with 0.4 N $HClO_4$ as previously described [21]. The NTPs in the neutralized acid-insoluble extract were analyzed by high pressure liquid chromatography using instruments from Waters Associates, Inc. (Milford, MA) equipped with two model 6000A pumps, and a Partisil 10 SAX (Whatman) anion-exchange column (250 × 4 mm). Quantitation of ribonucleotides and ara-CTP was conducted on an instrument equipped with a model 660 gradient programmer, a model 440 UV detector, and a model 730 data module. Deoxynucleotide determinations employed a model 490 UV detector and a model 840 data and chromatography control system.

Cellular NTPs and ara-CTP were separated by a concave gradient (curve 9) run over 30 min at a flow rate of 3 ml/min starting at an initial buffer composition of 65% buffer A (0.005 M $NH_4H_2PO_4$, pH 2.8) and 35% buffer B (0.75 M $NH_4H_2PO_4$, pH 3.5) and ending at 100% buffer B [21]. The amount of NTP (detected at 280 nm) was determined by external standard quantitation. The intracellular NTP concentration was calculated by dividing the NTP amount by the number of cells analyzed and the mean cell volume. This calculation was based on the assumption that the $HClO_4$-extracted nucleotides were distributed uniformly in cellular H_2O.

Determination of dNTP pools. The $HClO_4$-soluble neutralized cell extracts were evaporated to dryness in an Evapomix volume reduction apparatus (Buchler Instruments, Fort Lee, NJ). Degradation of ribonucleotides in the cell extract was achieved by periodate oxidation according to the method of Neu and Heppel [22]. Briefly, $HClO_4$-soluble material from 2×10^7 cells was incubated in the dark with 20 μmol $NaIO_4$ for 20 min at room temperature in a final volume of 700 μl. After addition of 200 μmol cyclohexylamine, the incubation was continued for a further 90 min at 45°. Glycerol (20 μmol) was added to the reaction, which was then incubated for 30 min at room temperature before the pH was adjusted to 6 with formic acid. The periodate-treated cell extracts were analyzed for dNTP content on the same day. Separation of dNTP and ara-CTP was achieved on a Partisil-10 SAX column. An isocratic elution with 75% buffer A (0.005 M $NH_4H_2PO_4$, pH 2.8) and 25% buffer B (0.75 M $NH_4H_2PO_4$, pH 3.7) was maintained for 20 min at a flow rate of 3 ml/min. Subsequently, a linear gradient led to 31% buffer A and 69% buffer B over 23 min. The elution was terminated after a total run time of 47 min. Retention times of nucleoside triphosphates were: dCTP, 31 min; ara-CTP, 34 min; dTTP, 36 min; dATP, 39 min; and dGTP, 45 min. The recovery of dNTP after the periodate oxidation was greater than 90% as indicated by material balance measurements of radioactive internal standards.

Determination of dCMPD activity. Extracts of CEM cells were prepared according to the method of Saunders and Lai [23]. Extracts were dialyzed against 50 mM phosphate buffer, pH 7.5, to deplete the preparations of endogeneous nucleosides and nucleotides. dCMPD activity was determined by the high pressure liquid chromatographic method reported by Fridland and Verhoef [24]. The production of dUMP was measured after 2-, 4-, and 6-min incubations at 37°, and the slope of the line was used to determine the reaction rate. The activity of dCMPD was expressed as nanomoles of dUMP produced per minute per milligram of protein, using bovine serum albumin as a standard.

RESULTS

Cellular metabolism of deoxycytidine. CCRF-CEM cells were exposed to 0.1 to 1000 μM dCyd for 2 hr. Within this range, dCTP and dTTP increased linearly with the dCyd concentration (Fig. 1). At a dCyd concentration of 1000 μM the dCTP pool increased from 24 to 75 μM, whereas the dTTP pool expanded from 47 to 140 μM. The failure to achieve a greater expansion of the dCTP pool was probably due to regulation of dCMP production caused by feedback inhibition of dCyd kinase by the enlarged dCTP pool [10, 11]. Similarly, the relatively constant ratio of dCTP to dTTP pools may also be attributed to the limited availability of dCMP for channeling by dCMPD through dUMP for dTTP synthesis. In contrast to the increase of the pyrimidine deoxynucleotides, dGTP rose only 34%, whereas dATP

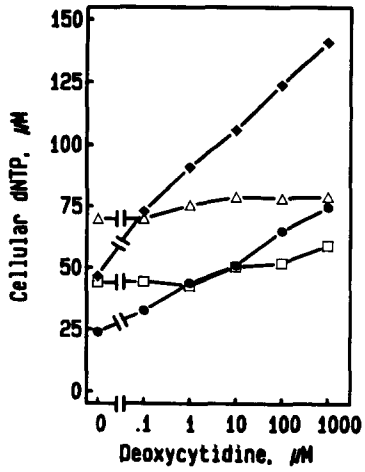

Fig. 1. Distribution of dCyd into dCTP and dTTP pools. CCRF-CEM cells were exposed to 0.1 to 1000 μM dCyd. After 2 hr of incubation, cellular dNTP concentrations were analyzed as described in Materials and Methods. Symbols: (●) dCTP; (◆) dTTP; (△) dATP; and (□) dGTP. The data are representative of three experiments.

Table 1. Effect of dTHU on the distribution of [^{14}C]dCyd in dNTP pools

Time of [^{14}C]dCyd addition (min)	Percent [^{14}C]distribution	
	dCTP	dTTP
0	56.0 ± 1.0	44.0 ± 1.5
10	73.0 ± 1.0	27.0 ± 1.0
20	95.0 ± 1.0	5.0 ± 1.0
30	97.0 ± 0.1	3.0 ± 0.7
45	98.5 ± 0.1	1.5 ± 0.1
60	98.7 ± 0.6	1.3 ± 0.5

CCRF-CEM cells were incubated with 100 μM dTHU. At the indicated times, a portion of the culture was pulsed with [^{14}C]dCyd (0.25 μCi/ml) for 10 min. The distribution of radioactivity in the dCTP and dTTP pools was determined as described in Materials and Methods. The time point at 0 min is the distribution of [^{14}C]dCyd in cells that had not been incubated with dTHU. Values are means ± SD, N = 3.

was not affected by the 2-hr incubation with 1000 μM dCyd.

Kinetics of dTHU-mediated dCMPD inhibition. CCRF-CEM cells express a high level of dCMPD activity [6, 24]. The apparent K_m and V_{max} values for dCMP in the presence of 5 μM dCTP were 63 ± 3 μM and 27.1 ± 2.4 nmol/min/mg protein respectively. The activity of dCMPD in whole CCRF-CEM cells was determined by pulse labeling cells with [^{14}C]dCyd and measuring the incorporation of radioactivity into the dTTP pool at various times during exposure to 100 μM dTHU (Table 1). The distribution of radiolabel between dCTP and dTTP pools was 56%:44% in control cells. Subsequently, pulse labeling of cells with [^{14}C]dCyd was undertaken at various times after addition of 100 μM dTHU to determine the effect on dCMPD. The incorporation of radiolabel into dTTP decreased to 5% 20 min after the start of dTHU incubation and to <2% after 45 min. These findings suggest that dTHU-induced dCMPD inhibition was a delayed process that neared completion after 45 min of incubation.

Effect of THU and dTHU on dNTP metabolism. Incubation of cells with dTHU resulted in a significant expansion of the dCTP pool (Fig. 2A). Within a dTHU concentration range of 3 to 100 μM, the dCTP pool expansion was directly proportional to the log of the dTHU concentration. dTHU is an effective inhibitor not only of dCMPD but also of CD [5]. It was possible to differentiate the effects of dTHU on dCMPD and CD with respect to dNTP metabolism by the use of THU, a specific inhibitor of CD. Cells incubated with up to 300 μM THU for 2 hr did not show any alteration of dNTP (Fig. 2B) or NTP concentrations (data not shown). Continued incubation for 6 hr with 100 μM THU failed to affect dNTP pools (data not shown). The effect of dTHU on dCTP metabolism appeared, therefore, to be associated specifically with inhibition of dCMPD.

The dCTP pool expansion reached its maximum after a 2-hr incubation with 100 μM dTHU (Fig. 3). This perturbation of the dCTP pool had no significant effect on the pool sizes of dTTP, dATP, or dGTP. The cellular NTP pools were unaffected (data not shown).

Reversibility of dTHU-mediated inhibition of dCMPD. Cells were incubated with 100 μM dTHU for 2 hr to expand dCTP pools. When washed into drug-free medium, the dCTP pool showed an immediate decrease (Fig. 4). Within 4 hr the cellular concentration of dCTP was reduced from 204 to 37 μM. The decrease of intracellular dCTP was linear over 4 hr. While dATP and dGTP pools remained unaffected, the dTTP pool had expanded more than 3-fold 4 hr after dTHU washout. The net decrease of the cellular dCTP concentration during this time interval was 157 μM compared with a dTTP net increase of 118 μM. Assuming that the dCTP that entered the dCMP pool by dephosphorylation was channeled to dTTP biosynthesis via dCMPD, there would have been more than an adequate amount of substrate to account for the observed increase in the dTTP pool.

Identification of the source of the expanded dCTP pool. In considering the role of the dCyd salvage pathway in the dCTP pool expansion, it is important to emphasize that these experiments were carried out in medium that contained dialyzed serum. Thus, exogenous dCyd should not have contributed to the observed expansion of the dCTP pool. It may be assumed, therefore, that the salvage pathway did not make a significant contribution to this effect.

As an alternative explanation, the participation of the *de novo* pathway of dCTP biosynthesis was analyzed (Table 2). Again, the marked expansion of the dCTP pool was observed in cells treated wtih dTHU. Inhibition of ribonucleotide reductase by 5 mM hydroxyurea alone resulted in an 80% decrease in dCTP pools but had lesser effects on dATP and dGTP pools. The dTTP pool was not affected by this treatment. Incubation of cells with dTHU in the presence of hydroxyurea completely prevented the dCTP pool expansion associated with dTHU treatment. This result indicates that the ribo-

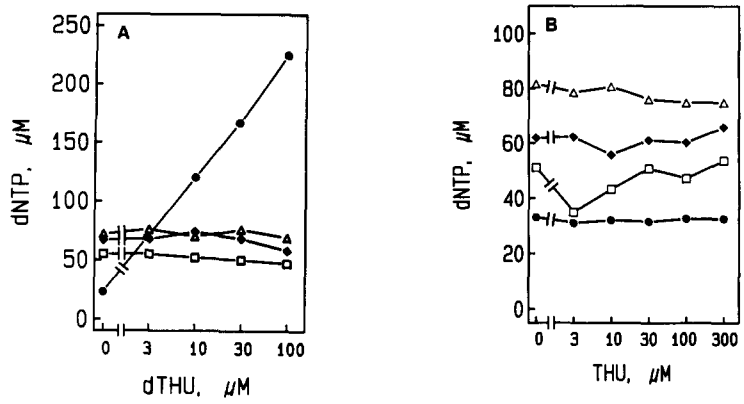

Fig. 2. Modulation of cellular dNTP pools by deaminase inhibitors. (A) Effect of dTHU on the dNTP concentrations in CCRF-CEM cells. Cells were incubated with dTHU (3–100 μM) for 2 hr, and then dNTP pools were extracted and analyzed as described in Materials and Methods. (B) Effect of THU on the dNTP concentrations in CCRF-CEM cells. Cells were incubated with 3–300 μM THU for 2 hr, and then cellular dNTP pools were analyzed as described in Materials and Methods. The data in each panel are representative of three experiments. Symbols: (●) dCTP; (◆) dTTP; (△) dATP; and (□) dGTP.

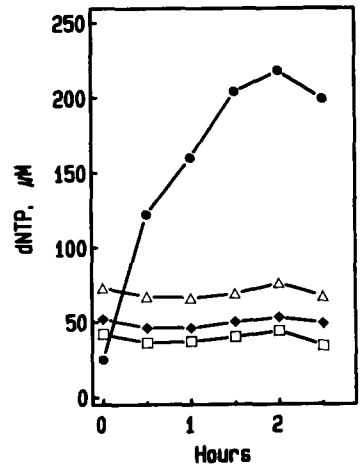

Fig. 3. Effect of dTHU on dNTP pools. Cells were incubated with 100 μM dTHU for the indicated times, and then cellular dNTP pools were analyzed as described in Materials and Methods. The data are representative of three experiments. Symbols: (●) dCTP; (◆) dTTP; (△) dATP; and (□) dGTP.

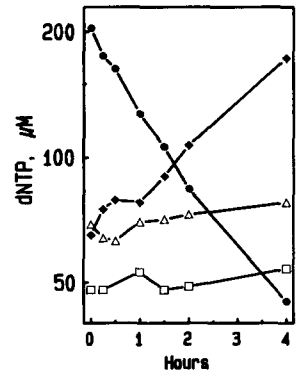

Fig. 4. Kinetics of dNTP pools after dTHU washout. CCRF-CEM cells were exposed concurrently to 100 μM dTHU and [5-^{3}H]dCyd (0.25 μCi/ml). After a 2-hr incubation, the cells were washed into drug-free medium. At indicated time points after drug washout, the cells were analyzed for intracellular deoxynucleotide concentrations as described in Materials and Methods. The data shown are representative of three experiments. Symbols: (●) dCTP; (◆) dTTP; (△) dATP; and (□) dGTP.

nucleotide pool is the major contributor of cytosine nucleotides to the dCTP pool expansion induced by dTHU.

Effect of an expanded dCTP pool on dCyd kinase activity. The activity of dCyd kinase is regulated by a feedback inhibition mechanism involving dCTP [10, 11]. To understand the impact of the dTHU-induced expansion of the dCTP pool on dCyd kinase activity, we determined the effect of dTHU on the cellular metabolism of ara-C, an alternative substrate for dCyd kinase. Cells were pulse labelled with 0.1 μM [^{3}H]ara-C during exposure to 100 μM dTHU. Since in the presence of high levels of ara-C dCyd kinase is the rate-limiting enzyme in the accumulation of ara-CTP [25, 26 and references therein], the radioactivity incorporated into the ara-CTP pool was used to estimate the activity of dCyd kinase (Fig. 5). Thirty minutes after dTHU addition, dCTP had reached a concentration of 143 μM. This was associated with a significant reduction of ara-CTP accumulation. At an intracellular dCTP concentration of 200 μM (2 hr) the phosphorylative activity was inhibited by 84% of control values. Thus, dCMPD inhibition induced a dCTP pool expansion that had a concentration-dependent effect on the activity of dCyd kinase. By comparison, when cells were pulsed with 100 μM [^{3}H]ara-C the activity of dCyd kinase was inhibited 68% when the dCTP concentration had reached 200 μM (2 hr). This suggests that even a 1000-fold increase of ara-C concentration was ineffective at overcoming the inhibition of dCyd kinase activity associated with the expanded dCTP pool.

Table 2. Effects of dTHU and hydroxyurea on dNTP pools in CCRF-CEM cells

	Cellular dNTP pool (μM)			
	dCTP	dTTP	dATP	dGTP
Control	26 ± 1	57 ± 4	82 ± 12	47 ± 1
dTHU, 100 μM	220 ± 13	70 ± 7	86 ± 7	46 ± 4
Hydroxyurea, 5 mM	5 ± 2	60 ± 7	57 ± 4	33 ± 5
dTHU, 100 μM and hydroxyurea, 5 mM	10 ± 4	55 ± 1	48 ± 11	28 ± 5

Cells were incubated for 2 hr with 100 μM dTHU alone, 5 mM hydroxyurea alone, or a combination of both drugs. Intracellular dNTP concentrations were determined as indicated in Materials and Methods. Values are means ± SD, N = 3.

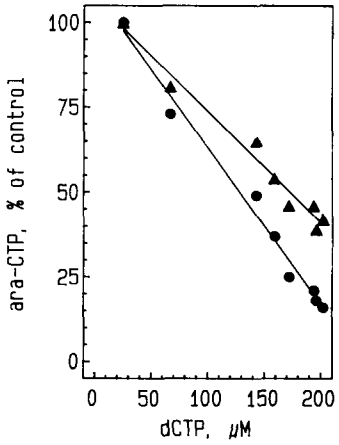

Fig. 5. Effect of dTHU-induced dCTP pool expansion on ara-CTP accumulation. CCRF-CEM cells were exposed to 100 μM dTHU for up to 120 min to allow dCTP pools to expand as indicated. At 0 (no dTHU), 15, 30, 45, 60, 75, 90, and 120 min, aliquots of cells were pulsed for 10 min with either 0.1 μM (●) or 100 μM (▲) [^{3}H]ara-C. At the end of the pulse, nucleotide pools were extracted and analyzed for [^{3}H]ara-CTP and dCTP content. The cellular ara-CTP concentration, expressed as a percent of the ara-CTP in cells not incubated with dTHU, was compared with the cellular concentration of dCTP at the end of the pulse of each aliquot of cells. A value of 100% was equal to 0.5 and 38 μM intracellular ara-CTP in cells that had been pulsed with 0.1 and 100 μM ara-C respectively.

DISCUSSION

The present study provides insight into the regulation of deoxycytidine nucleotide metabolism in intact cells. It is clear that the activity of dCyd kinase is regulated by dCTP [10, 11], and that cellular dCTP pools are unlikely to control the rate of dCMP accumulation via the salvage pathway in whole cells [25]. Although human plasma levels of dCyd are in the range of 0.5 to 4 μM [27], the cellular dCTP pools were increased barely 3-fold by dCyd concentrations 1000-fold greater (Fig. 1). This suggests that the salvage pathway is unlikely to contribute greatly to an expansion of dCTP pools. Nevertheless, this modest increase in dCTP levels did lead to an expansion of the dTTP pools 2-fold greater than dCTP, consistent with the role of dCMPD in sustaining the synthesis of thymidylate [8, 9].

The importance of dCMPD to dCTP metabolism in CCRF-CEM cells became apparent when dTHU was used to inhibit the enzyme. Inhibition of dCMPD, which was dependent upon both dTHU concentration and time of incubation, increased cellular dCTP as much as 8-fold without addition of exogenous dCyd (Figs. 2A and 3). Although dTHU is known to inhibit CD [5], the lack of a similar effect by the specific CD inhibitor THU (Fig. 2B) and the fact that CCRF-CEM cells are deficient in CD [24] support the conclusion that the dCTP pool expansion was due to inhibition of dCMPD alone.

A 97% inhibition of dCMPD was achieved after 45 min of incubation with 100 μM dTHU (Table 1). The rather slow onset of dTHU-mediated dCMPD inhibition may be explained by the fact that phosphorylation of dTHU to dTHUMP is necessary to induce dCMPD inhibition [5, 6]. Moran *et al.* [6] reported a relatively low phosphorylation rate (1.8×10^{-3} pmol/min/mg) for dTHU by CCRF-CEM cell extracts. It is unclear, however, which enzyme is responsible for phosphorylation of dTHU.

The dCTP pool expansion was linear with dTHU concentration (range, 3 to 100 μM) and reached a maximum (≥200 μM) after a 2-hr exposure to 100 μM dTHU (Fig. 2A). It would be expected that dCMP would accumulate behind a metabolic block at dCMPD. The activity of pyrimidine nucleoside monophosphate kinase (EC 2.7.4.14) has been reported to be high in cells [14, 15], although the enzyme exhibits a relatively low affinity for dCMP [8]. Under conditions where the dCMP concentration is the rate-limiting factor for dCMP phosphorylation, inhibition of dCMPD would be expected to result in an increased rate of dCMP phosphorylation. Thus, the dCTP pool expansion may be seen as a result of the accelerated phosphorylation of excess dCMP [8] in the absence of dCMPD activity, which normally curtails the cellular concentration of this substrate.

The inhibition of dCMPD did not affect the size of the dTTP, dATP, or dGTP pools (Fig. 2A). NTP pools remained unchanged, and growth inhibition was not observed as a result of dTHU-mediated dCMPD inhibition. Several laboratories [18–20] have reported a 30–50% decrease in dTTP pools, which became limiting to growth in the absence of exogeneous thymidine in two dCMPD-deficient hamster fibroblast cell lines. We would expect dTHU to induce a similar requirement in cell cultures maintained in the presence of the inhibitor. The experiments reported here, however, were conducted over

2–4 hr, a duration of less than 20% of the population doubling time during which significant changes in the dTTP pool might not be expected. Additionally, dTTP concentrations may be maintained through increased use of dUMP derived from UDP reduction. Parallel incubation of CCRF-CEM cells with hydroxyurea and dTHU resulted, however, in only an insignificant decrease of dTTP. Moreover, since experiments were performed in dialyzed serum, uptake of dUrd and dThd from the medium appears less probable as the source of dTTP maintenance.

The inhibition of dCMPD by dTHU appeared to be reversible. The linear decrease of the dCTP pool (Fig. 4) after washout of 100 μM dTHU can be interpreted as a resumption of dCMPD activity, although utilization of dCTP for DNA synthesis may also be expected to deplete the expanded pool. This observation may indicate that the intracellular dTHUMP concentration was subjected to a rapid metabolism once dTHU was removed. It is likely that only relatively low cellular concentrations of dTHUMP were required to evoke inhibition of dCMPD. Maley and Maley [5] determined a K_i of $1.0–2.0 \times 10^{-8}$ M and a $K_m : K_i$ (dCMP : dTHUMP) of $3.0–6.0 \times 10^{-4}$ in the chick embryo mince system. Thus, a substantial inhibition of dCMPD may be affected by low cellular concentrations of dTHUMP which, in the absence of exogenous dTHU, could be rapidly metabolized, leading to prompt reversal of enzyme inhibition. The cellular pharmacodynamics of dTHUMP remains a topic for future investigations.

The decrease of the dCTP pool after dTHU washout was accompanied by a significant elevation of the dTTP pool (Fig. 4), indicating that dCMPD activity may be rate limiting for dTTP production. It appears that the rate of dCMP deamination is greater than the velocity of dCMP dephosphorylation. Thus, most of the dCMP entering the dCMP pool either via dCTP dephosphorylation of through ribonucleotide reductase activity is subjected to deamination by dCMPD.

The dTHU-induced dCTP pool expansion was completely prevented by coincubation of CCRF-CEM cells with 100 μM dTHU and 5 mM hydroxyurea (Table 2); it may be considered a function of ribonucleotide reductase activity. According to the model of allosteric regulation of ribonucleotide reductase [12, 13, 28], dCTP has no regulatory influence on the activity of the enzyme. The dCTP pool expansion through ribonucleotide reductase may, therefore, occur independent of the cellular dCTP concentration. Thus, the activity of dCMPD and the pools of effectors of ribonucleotide reductase regulate the dCTP concentration in intact cells.

The cellular activity of dCMPD may be of importance with regard to the metabolism of ara-C [24, 29] and possibly other clinically active cytosine nucleoside analogues [30–32]. Although ara-CMP appears to be a poor substrate for dCMPD [17, 33], the higher specific activity of the enzyme in human leukemia cells relative to normal tissues may be an important determinant of the cellular pharmacology of ara-C nucleotides [24, 29]. Investigations of the metabolism of ara-C triphosphate in cells treated with dTHU may aid evaluations of the role of dCMPD in the action of ara-C.

On the other hand, dCMPD deficiency is associated with an increase in dCTP pools, a factor implicated in resistance to ara-C [18, 34]. dCTP exerted a significant feed-back inhibition on dCyd kinase and thus decreased ara-C phosphorylation to the active triphosphate (Fig. 5). The dTHU-mediated expansion of the dCTP pools may provide the pharmacologic phenotype of this condition. It is possible that this type of resistance to ara-C may be overcome by inhibitors of ribonucleotide reductase such as hydroxyurea. dTHU may, therefore, be a compound that can be used clinically to modulate the cellular metabolism of dNTPs and of ara-C.

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