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Compartmentation of dCTP pools disappears after hydroxyurea or araC treatment in lymphocytes

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The calculated rate of DNA synthesis using [5-^{3}H]TdR was about 4 times higher than in the case of [5-^{3}H]CdR labeling, even after correction for the specific radioactivities of the intracellular pools. These data show a compartmentation of dCTP pools in lymphocytes. Hydroxyurea increased the specific activities of both dTTP and dCTP pools so that the calculated rate of DNA synthesis became equal. The same effect was found for araC treatment, but not for fluorodeoxyuridine. dCTP was supplied from CTP which is the lowest ribonucleotide pool in lymphocytes. Different functions of the two dCTP pools are proposed: one serving DNA replication; the other one supplies phospholipid precursors and DNA repair.

Deoxycytidine; Pool compartment; Phospholipid; Hydroxyurea; araC; Lymphocyte

1. INTRODUCTION

Autoradiography of lymphoid tissues shows a different picture if labeled either by radioactive thymidine or by radioactive deoxycytidine [1,2]. Isolated lymphocyte subpopulations also vary in the sufficiency for incorporation of these labeled precursors into DNA [3]. The explanation of these differences is ambiguous.

On the other hand, compartmentation of dCTP was suggested by Nicander and Reichard [4] and further evidence in fibroblasts was supported by Spyrou and Reichard [5,6]. The function of the two distinct dCTP pools seemed to be related to a new pathway of dCTP: in addition to DNA synthesis, it can also serve phospholipid synthesis through the production of deoxyliponucleotides like dCDP-choline and dCDP-ethanolamine [6–8]. Compartmentation of dCTP in relation to the synthesis not only of water-soluble deoxyliponucleotides, but also lipidic dCDP-diacylglycerol, was shown in our laboratory [7–10].

In this paper we present further evidence for distinct dCTP pools in human lymphnode lymphocytes. The effect of inhibitors like arabinosyl-cytosine (araC), hydroxyurea (HU), and 5-fluoro-deoxyuridine (FdU) was also investigated, leading to some conclusions about the function of dCTP compartments.

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2. METHODS

2.1. *Cells*

Human tonsillar lymphocytes were prepared from infant tonsils as described [3]. Short-term cultures were labeled with either [5-^{3}H]CdR (740 GBq/mmol) or with [5-^{3}H]TdR (888 GBq/mmol) for 40 min in Eagle's MEM in the presence or absence of 1 mM hydroxyurea, 1 μM araC or 1 μM 5-fluorodeoxyuridine.

2.2. *Separation of deoxyliponucleotides*

Acid-soluble fractions of cells were neutralized and used for HPLC separation on a Partisil-10-SAX column as described earlier [8].

2.3. *Measurement of specific radioactivity of dCTP and dTTP pools*

This was carried out according to Skoog [11] with the modification of direct measurements for the specific radioactivity of ribonucleoside triphosphate pools [12].

2.4. *Ribonucleoside triphosphate pools*

These pools were determined by RNA polymerase test [13].

3. RESULTS

3.1. *Salvage of CdR traps in two forms after hydroxyurea treatment: in dCDP-choline and in dCTP*

Short-term cultures of tonsillar lymphocytes were labeled with [5-^{3}H]CdR in the presence of different inhibitors, and the acid-soluble pool was analyzed with HPLC. In the presence of 5-fluorodeoxyuridine (FdU), a high accumulation of radioactivity in dUMP was found, as FdU inhibits the conversion of dUMP to dTMP. No other extensive accumulation was found during FdU treatment. However, in the presence of hydroxyurea (HU), radioactivity accumulated in dCTP,

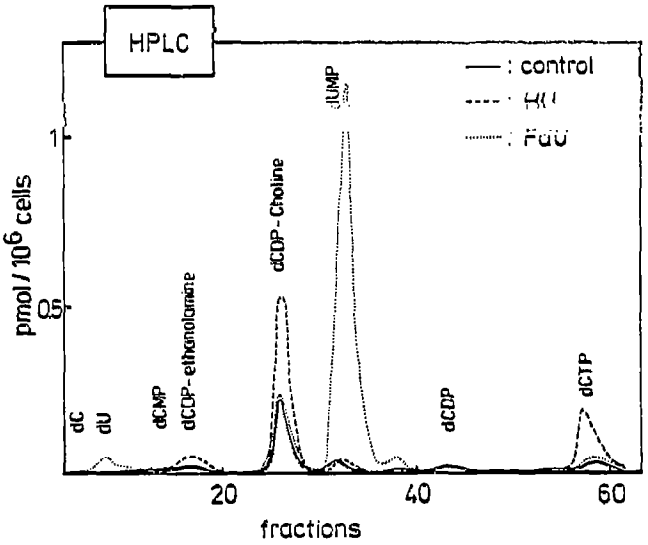

Fig. 1. Effect of hydroxyurea and fluorodeoxyuridine on the [^{3}H]CdR labeling of deoxyliponucleotides and deoxynucleotides in lymphocyte extracts. Tonsillar lymphocytes were labeled with [5-^{3}H]CdR for one hour in the presence of 1 mM hydroxyurea (---), or 1 μM 5-fluorodeoxyuridine (···), or without any inhibitor (———). Acid-soluble extracts of cells were neutralized and the labeled compounds were separated on HPLC and measured for radioactivity. Specific activity of extracellular [^{3}H]CdR was used for the calculation of pmoles.

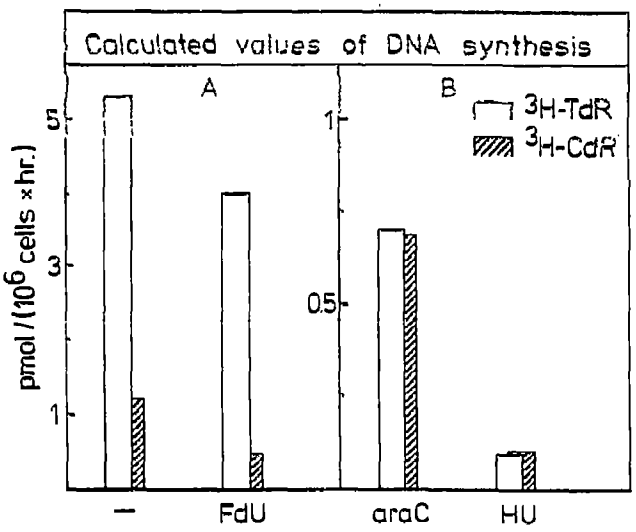

Fig. 2. Effect of FdU, araC and HU on the calculated rate of DNA synthesis. Lymphocytes were labeled either with [5-^{3}H]CdR (hatched columns) or with [5-^{3}H]TdR (open columns) in the presence of 1 μM fluorodeoxyuridine (A), 1 μM araC, or 1 mM hydroxyurea (B), or without any inhibitor (A). DNA synthesis expressed in pmol nucleotides incorporated per 10^6 cells during 1 h. These values were calculated from incorporated cpm into DNA and from the measured intracellular specific radioactivity of dCTP pools or dTTP, respectively (see Table I).

in accordance with its inhibitory effect on DNA precursor synthesis. Moreover, HU caused a pronounced accumulation of radioactivity in dCDP-choline, a supposed intermediate in phospholipid metabolism too, in spite of any known effect of HU on lipid metabolism (Fig. 1).

3.2. *CdR and TdR incorporate with different apparent rates into DNA*

In lymphocytes, [5-^{3}H]CdR incorporation into DNA is 2- to 4-fold lower than [^{3}H]TdR incorporation [7], suggesting a big pool size of dCTP compared to dTTP. However, it was not supported by direct measurement of the deoxypyrimidine nucleotide pools (Table I). Even after correction of incorporation of these precursors by the specific radioactivity of their intracellular pools, we observed wide differences in the rate of DNA synthesis for the same cell cultures (Fig. 2A). The calculated rate of DNA synthesis is 3–4 times higher when measured with [^{3}H]TdR than with [^{3}H]CdR. Calculation is based on the assumption that the total pool size, measured by the DNA-polymerase test is the only pool, i.e. no compartmentalization takes place. The same type of discrepancy was found in the presence of FdU (Fig. 2A). However, in the presence of araC or HU, the calculated rate of DNA synthesis was the same measured either with [^{3}H]TdR or with [^{3}H]CdR (Fig. 2B).

Table I

Effect of 5-FdU, araC and HU on dCTP and dTTP pools

		–	5-FdU	araC	HU
Pool size	dCTP	1.7	1.8	2.0	1.4
pmol/10^6 cells	dTTP	1.8	1.2	1.9	2.1
Spec. radioact.	dCTP	1247	1153	1165	4550
cpm/pmol	dTTP	1587	3227	1093	5173

3.3. *Comparison of S phase and G_1 phase lymphocytes*

Tonsillar lymphocytes were separated on an albumin gradient according to their density, as blast cells are supposed to have a low density (LD cells) compared to small, resting lymphocytes (HD cells). HD cells (10^6) contain 0.8–1.6 pmol of either dCTP or dTTP, respec-

Table II

Calculated rate of DNA synthesis in S (LD) and G_1 (HD) phase lymphocytes

		Tonsil 1		Tonsil 2	
		LD	HD	LD	HD
Pool size	dCTP	3.07	1.19	3.34	1.38
pmol/10^6 cells	dTTP	1.63	0.84	4.13	1.57
Spec. radioact.	dCTP	961	1430	1066	1173
cpm/pmol	dTTP	2325	2839	1860	2252
Rate of DNA synth.	[5-^{3}H]dC	2.55	0.55	3.16	1.29
pmol/h	[^{3}H]dT	5.18	1.65	11.63	3.57

Table III

Ribonucleoside triphosphate pools in tonsillar lymphocytes

	CTP	UTP	GTP	ATP
pmol/10^6 cell	23	66	78	240
Range	12–48	60–150	60–150	210–900

tively, and both pool sizes increase in blast cells showing a wild variation (1.6–4.2 pmol) according to the level of in vivo stimulation (Table II, 1st column). However, no relation was found between the specific radioactivity of these pools and the degree of stimulation (Table II). The calculated rate of DNA synthesis was found to be 2–4 times higher in any lymphocyte subpopulation, when the cells were labeled by [^{3}H]TdR compared to [^{3}H]CdR.

3.4. *Ribonucleotide pool sizes of lymphocytes*

As the intracellular precursors for 'de novo' deoxyribonucleotide synthesis are ribonucleotides, their pool sizes were also measured in these cells (Table III). The CTP pool is much smaller than any other ribonucleoside triphosphate pool, indicating that these cells do not have a large supply for 'de novo' dCTP synthesis.

4. DISCUSSION

Lymphocytes can be differently labeled by extracellular [^{3}H]CdR and [^{3}H]TdR, using either autoradiography [1,2] or other techniques [3]. Measurement of the pool sizes did not explain this phenomenon, and the calculated rate of DNA synthesis (based on the measured incorporation into DNA and specific radioactivity of precursor pools) was also different when the same cell culture was labeled either by [^{3}H]TdR or by [^{3}H]CdR (Tables I and II).

Searching for the reason of this discrepancy, it could be supposed that TdR and CdR incorporate in different ratios into DNA. However, human DNA does not differ more than 30% in respect to the ratio of nucleotides, and we measured a 300% difference. Another explanation might be that our cells were starving for TdR but not for CdR, and as soon as they were supplied with a trace amount of TdR (0.3 μM), DNA synthesis was stimulated. However, TdR does not have any effect at this low concentration (data not shown). Similarly, an inhibitory effect of CdR on DNA synthesis might explain the measured lower rate of DNA synthesis in the case of [^{3}H]CdR labeling, however, we did not find any inhibitory effect of trace amounts (0.3 μM) of CdR on [^{3}H]TdR incorporation. These experiments show that the rate of DNA synthesis must be the same, and to explain the results, compartmentation of the dCTP pool has to be considered (Fig. 3).

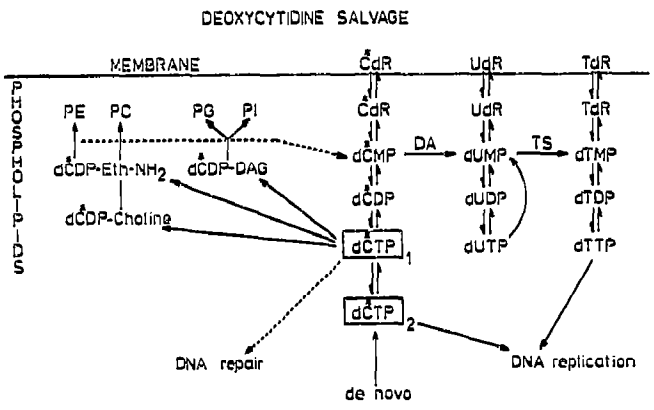

Fig. 3. Proposed model of common pathway for deoxynucleotides and phospholipids. CdR, deoxycytidine; UdR, deoxyuridine; TdR, deoxythymidine; PE, phosphatidyl-ethanol-amine; PC, phosphatidylcholine; PG, phosphatidyl-glycerol; PI, phosphatidyl-inositol.

The effect of different inhibitors (used as chemotherapeutic drugs) on the dCTP pool was also investigated. Compartmentation still exists in the case of 5-FdU treatment (Fig. 2A), but apparently disappears in the presence of araC and HU (Fig. 2B). In the case of HU, a plausible explanation is that inhibition of 'de novo' synthesis produces an undetectable $dCTP_2$ pool, and any kind of DNA synthesis (DNA repair) still operating, uses only $dCTP_1$. Similar pool changes were found for hydroxyurea in fibroblasts [14–16]. The effect of araC is more complicated as it does not directly inhibit the synthesis of deoxynucleotides. However, a strict regulation between replicative DNA synthesis, inhibited by araC, and the 'de novo' synthesis of deoxynucleotides can be considered.

What can be the function of the two dCTP pools? One of them is clearly supplied by 'de novo' synthesis and serves DNA replication ($dCTP_2$). The question is rather the function of the $dCTP_1$ pool, belonging to the salvage pathway, and serving deoxyliponucleotide synthesis, as suggested before [4–10]. This paper shows evidence for another possible function of this dCTP pool: 'non-replicative' or 'non-S-phase' DNA synthesis can be supplied from this pool. This can be repair of DNA, generally measured in the presence of HU [17], causing a decrease in the dATP pool, and consequently an increase in DNA repair. On the other hand, FdU increases dATP [18], inhibiting DNA repair, which is in accordance with our results that in the presence of FdU, compartmentation of the dCTP pool still exists (Fig. 2). However, any other kind of 'non-S-phase' DNA synthesis, taking place during lymphocyte differentiation [19,20] can also use the salvage pool of dCTP [19,20]. The question, whether there is a single connection between deoxyliponucleotide synthesis and 'non-S-phase' DNA synthesis; i.e. that they share a common precursor pool, remains open.

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