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Selective activation of deoxycytidine kinase by thymidine-5′-thiosulphate and release by deoxycytidine in human lymphocytes

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Abstract

Deoxycytidine kinase (dCK) catalyses the rate-limiting step of the salvage of three natural deoxyribonucleosides as well as several therapeutic nucleoside analogues, which in turn can enhance its enzymatic activity [Biochem Pharmacol 56 (1998) 1175], improving the efficacy of the cytostatic therapy. Here, we measured the effect of the 5′-thiosulphate (5′-TS) derivatives of four deoxyribonucleosides (deoxyadenosine, deoxycytidine (dCyd), azidothymidine, thymidine) and two ribonucleosides (ribopurine, ribouridine (Urd)) on the activity of the two main salvage deoxynucleoside kinases, and on the salvage of dCyd and deoxythymidine (dThd). It turned out that only 2′-deoxythymidine-5′-thiosulphate (dThd-5′-TS) can potentiate the dCK activity, without influencing the thymidine kinase isoenzymes during short-time treatments of human peripheral blood and tonsillar lymphocytes. The enhancement of dCK activity by dThd-5′-TS can be reversed by dCyd, but dThd had no effect on the enzyme activation in cells. Neither dThd-5′-TS nor Urd-5′-TS had any effect on the dCK and thymidine kinase activities tested in cell-free extracts. The stimulation of dCK activity in cells was accompanied by an imbalance in the dThd and dCyd metabolism. The incorporation of ^{3}H-dThd into DNA was suppressed by 90% in cells by dThd-5′-TS, while Urd-5′-TS only slightly influenced the same process. The ^{3}H-dCyd incorporation into DNA was inhibited only to 50% of the control, while the ^{3}H-dCyd labelling of the nucleotide fraction was enlarged in dThd-5′-TS-treated cells, as a consequence of the increased dCK activity. We suggest that the enhancement of dCK activity is a compensatory mechanism in cells that might be induced by different ‘‘inhibitors’’ of DNA synthesis leading to damage of DNA. The increased dCK activity is able to supply the repair of DNA with dNTPs in quiescent cells; this suggestion seems to be supported by the counteracting effect of extracellular dCyd, too.

Keywords: Nucleoside-5′-thiosulphates; Deoxycytidine kinase; Thymidine kinases; Deoxynucleoside salvage; Enzyme modulation; DNA repair

1. Introduction

dCK (EC 2.7.1.74) plays a pivotal role in the mammalian nucleoside salvage pathways by recycling deoxynucleosides either released from the intracellular DNA catabolism or provided via nucleoside-specific membrane transporters from the extracellular environment [1]. This enzyme is responsible for the first and rate-limiting 5′-phosphotransfer from ATP or UTP [2] to 2′-deoxycytidine and to the two purine deoxynucleosides [2]. Moreover, based on its surprisingly broad substrate specificity regarding chemical modifications occurring either in the base or sugar moieties, dCK efficiently phosphorylates and thereby activates a wide range of nucleoside analogues, fundamental components of antileukemic, antitumour and antiviral therapeutic regimens [3,4]. The most widely used antimetabolites are: 2-chloro-2′-deoxyadenosine (Cladribine) [3], 2′,2′-difluorodeoxycytidine (Gemcitabine) and 1-β-D-arabinosylcytosine as antiproliferative agents [3] or 3′-azido-2′,3′-dideoxythymidine

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Abbreviations: dCK, deoxycytidine kinase; TK, thymidine kinase; ^{3}H-dCyd, 2′-deoxy-[5-^{3}H]cytidine; ^{3}H-dThd, 2′-deoxy-[5-methyl-^{3}H]thymidine; CdAdo, 2-chloro-2′-deoxyadenosine; dThd-5′-TS, 2′-deoxythymidine-5′-thiosulphate; dAdo, 2′-deoxyadenosine; rPu, ribopurine; Urd, uridine; dCMP, 2′-deoxycytidine-5′-monophosphate; HIV, human immunodeficiency virus; PBS, phosphate buffered saline; DEAE, diethylaminoethyl; dNTP, deoxynucleoside triphosphate; TS, thiosulphate.

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and (*S*,*S*)-isodideoxyadenosine as powerful compounds against HIV infection [4,5].

dCK is located in the proximity of the cytoplasmic membrane as well as in the perinuclear area [6] and shows a constitutive expression throughout the cell cycle [6–8], although 2- to 3-fold differences in its activity have been reported in different phases of the cell cycle [8]. Especially in resting cells, such as G_0/G_1 phase lymphocytes, thymocytes and splenocytes and central neurons, its contribution to DNA repair and membrane liponucleotide synthesis is indispensable [9,10]. Some solid tumours also express dCK at high levels; a positive correlation has been verified between dCK enzyme activity and the sensitivity of malignant tissues towards chemotherapy [11]. Resistance to nucleoside analogues has been attributed to impaired dCK function implying various exon deletions and alternatively spliced isoforms [12,13]. Antisense oligonucleotides and retroviral introduction of hammerhead ribozymes have been reported to result in compromised dCK activity [14,15]. Remarkably, full sensitivity has been restored upon transfection of the dCK cDNA into resistant cell lines [16].

Previously we have found that upon short-term treatments of human primary lymphocyte cultures with the above mentioned nucleoside analogues, dCK activity has elevated several times coinciding with the inhibition of DNA synthesis, without any increase in the mRNA and protein levels of the enzyme [17]. The same phenomenon occurred upon preincubation with a variety of non-nucleoside genotoxic agents, such as aphidicolin [18], etoposide [19,20], taxol and even with the G-protein modulator sodium fluoride [21]. Opposite results were obtained with protein phosphatase inhibitors [18], pointing to a presumable regulatory role of reversible protein phosphorylation during the short incubation period. These findings have a high impact on the implementation of combined chemotherapy: administration of a given drug – even with no structural homology towards nucleoside analogues – potentiates the utilisation of the nucleoside analogues *via* the enhancement of dCK activity. Gamma-irradiation also augments dCK function [22], and some recent papers also corroborate these findings, which also show that the sequence of treatments is also important [11,23–25].

Aliphatic thiosulphates (also known as Bunte salts, [26]) possess antibacterial and fungicidal activity [27]. A number of sulphur precursors can undergo metabolic oxidation to generate sulphane compounds which inhibit both 2′-deoxy-[5-methyl-^{3}H]thymidine (^{3}H-dThd) incorporation and the rate of proliferation in a human hepatoma cell line. However, treatment with thiosulphates has not elicited significant changes in this context [28].

In the present study we investigated the effect of several nucleoside-5′-TS derivatives on the level of the two main deoxynucleoside kinases and on their salvage processes in cells. Primary cultures of human tonsillar lymphocytes and peripheral blood lymphocytes were incubated in the presence of these compounds in short-term treatments. After washing and harvesting the cells, dCK and thymidine kinase (TK) activities were measured in the crude cell extracts. The metabolism of labelled pyrimidine nucleosides was also determined in the pools and in DNA. Out of the six investigated purine- and pyrimidine-5′-TS derivatives, only thymidine-5′-TS triggered significant alterations in the salvage routes, namely dCK activity elevation and severe suppression of thymidine uptake and incorporation into DNA, without influencing the TK activities.

2. Materials and methods

2.1. Chemicals

Nucleoside-5′-TSs were synthesised by Kazimierczuk and co-workers [26]. 2′-Deoxy-[5-^{3}H]cytidine (^{3}H-dCyd; specific activity: 16.2 Ci/mmol, 37 MBq/mL) and ^{3}H-dThd (specific activity: 25 Ci/mmol, 37 MBq/mL) were purchased from Amersham Life Sciences. Non-labelled nucleosides were obtained from Sigma Chemical Co. All the other chemicals were of analytical grade and produced by Reanal.

2.2. Cell cultures, drug treatments and preparation of cell-free extracts

Human tonsillar lymphocytes were freshly isolated from surgically removed tonsils of 3–6-year-old children. Cells were washed twice and resuspended in serum-free Eagle's minimal essential medium (MEM); concentration was adjusted to 10^7 cells/mL. Primary cell cultures were incubated at 37° in the presence or absence of different analogues for short-time periods (1–3 hr), as described earlier [17,29]. Treatments were stopped by washing the cells twice with ice-cold PBS.

Crude cell extracts were prepared by three consecutive freeze–thaw cycles (liquid nitrogen–ice) in extraction buffer containing 50 mM Tris–HCl, pH 7.6, 2 mM dithiothreitol (DTT), 0.5 mM phenylmethylsulphonyl fluoride (PMSF), 20 v/v% glycerol and 0.5% Nonidet P-40 nonionic detergent. After centrifugation for 30 min at 20,600 *g* (Hettich, Universal 32 R) at 4°, supernatants with an average protein concentration of 5 mg/mL were subjected to dCK and TK enzyme activity determinations.

Peripheral blood mononuclear cells (PBMCs) were obtained from healthy human donors, separated on Ficoll-Hypaque (Pharmacia) density gradient, and treated in the same way as tonsillar lymphocytes.

2.3. dCK and TK enzyme assays

dCK and TK activities were measured using ^{3}H-dCyd and ^{3}H-dThd as substrates (both 10 μM; specific activities:

500–1000 cpm/pmol), respectively, in a kinase assay buffer containing 50 mM Tris–HCl, pH 7.6, 5 mM $MgCl_2$, 5 mM ATP, 2 mM DTT, 10 mM NaF and as much cell extract as to be optimal for the reaction to proceed in the linear kinetic range, assessed during preliminary experiments. To stop the reaction, aliquots were spotted onto diethylaminoethyl (DEAE)–cellulose filters (less than 10% of binding capacity was used), which were subsequently washed, eluted and radioactivity was counted as described [29]. TK2 isoenzyme was measured in the presence of 1 mM dCyd in the TK reaction mixture [29]. TK2 activity was less than 1% of total TK activity in our cells and did not change during treatments.

The indicated analogues were added directly to dCK or TK enzyme assays to test their effects on kinase activities *in vitro* too. Parallel samples were incubated for 30–60 min, and enzyme assays were commenced immediately, as described beforehand.

2.4. Metabolism of labelled deoxynucleosides

Lymphocytes were pulse-labelled either with ^{3}H-dCyd or ^{3}H-dThd (1.0 μCi/mL/10^7 cells, for 20 min at 37°), subsequently to the treatments of cells with the indicated analogues. Extracellular isotopes were removed by washing the cells three times with PBS, then cells were lysed in 70% ethanol at −20° overnight. Ethanol-soluble and -insoluble fractions were separated by centrifugation. The non-phosphorylated and phosphorylated labelled nucleosides were separated on DEAE–cellulose sheets as detailed in Refs. [30,31]. The ethanol-insoluble fraction was re-extracted with 0.5 N perchloric acid and the pellet was hydrolysed at 90°for 1 hr, followed by a toluene-based scintillation counting to determine the isotope incorporated into DNA [31].

3. Results

3.1. The effect of different nucleoside-5′-TSs on the level of dCK and TK activities in human tonsillar lymphocytes and in cell-free extracts

Different drugs have been shown to increase the activity of dCK during 2–3 hr treatments [17–22]. Many of them are nucleoside analogues bearing minor substitutions in the sugar and/or base moieties, used as cytostatic drugs. Here, we are investigating whether nucleoside-5′-TS analogues, that do not possess a free phosphoacceptor 5′-hydroxyl group, could also exert any effect on the main deoxynucleoside salvage pathway. The monoanionic 5′-TSs do have biological activity and can partially mimic the biochemical functions of their corresponding 5′-monophosphate congeners [27,28].

Primary cultures of human tonsillar lymphocytes were treated for 2 hr with 0.1 and 1.0 mM 2′-deoxyadenosine (dAdo-), dCyd-, ribopurine (rPu-), riboUrd-, 3′-azidothymidine (3′-azido-Thd-) and dThd-5′-TSs. The dCK activity values measured in cell-free extracts are depicted in Fig. 1A. Out of the six compounds investigated, only the thymidine derivative, dThd-5′-TS, resulted in a significant dCK enzyme activation (40% increase at 100 μM and 100% at 1 mM, compared to the untreated activity: 5.2 pmol/10^6 cells/min) already after 2-hr treatment. The rest of the analogues slightly decreased the activity as compared to the untreated control. The levels of TK isoenzyme activities were not significantly altered under the same experimental conditions (data not shown). Time and concentration dependence of dCK activation in the same experiment were also explored. Treatment with dThd-5′-TS resulted in a marked dCK activation even after 60-min incubation (1.2-fold at 0.1 mM and 1.7-fold at 1.0 mM, respectively). However, the effect became more pronounced after 2 hr (1.45- and 2.20-fold, respectively) as shown in Fig. 1B.

The influence of dThd-5′-TS and Urd-5′-TS on the enzyme activity was tested directly and it was revealed that the augmentation of the salvage enzyme dCK was strictly confined to the unimpaired cellular context. When cell-free extract was prepared from control cells and the reaction mixture was subsequently complemented with 0.1 and 1.0 mM concentrations of dThd-5′-TS or Urd-5′-TS and incubated for 30 and 60 min, no significant change was observed either in dCK, or in TK activities. Note that in this case the analogue was present during the whole time period of activity measurement (Fig. 1C).

3.2. The release of the enhancing effect of dThd-5′-TS by dCyd but not by thymidine both in PBMCs and lymph node lymphocytes

The enhancement of dCK activity by dThd-5′-TS has also been tested in PBMCs, as the activation of dCK was shown earlier in different cell lines [19]. As can be seen in Fig. 2, dCK activity elevated from 3.8 to 8.8 pmol 2′-deoxycytidine-5′-monophosphate (dCMP)/10^6 cells/min in dThd-5′-TS (1 mM)-treated PBMCs (blank bars), while TK1 activity did not change during the 2-hr treatment by the dThd-5′-TS (striped bars). It turned out that dCyd at 100 μM concentration is a very potent inhibitor of the dThd-5′-TS-induced dCK activation process in PBMCs. It decreased both the control and the intensified dCK activity to the same level: from 3.8 pmol/10^6 cells/min to 2.2 and from 8.8 to 2.2, respectively. The counteracting effect of both pyrimidine deoxynucleosides was tested in tonsillar lymphocytes too, with respect to the two corresponding kinase (i.e. dCK and TK) activities. Similarly to the results obtained with PBMCs, only dCyd but not dThd was able to rescue the enhancement of dCK activity induced by dThd-5′-TS in tonsillar lymphocytes (Fig. 3). We completed the medium (containing 100 μM or 1 mM dThd-5′-TS) with 100 μM dThd, but could not see any significant influence

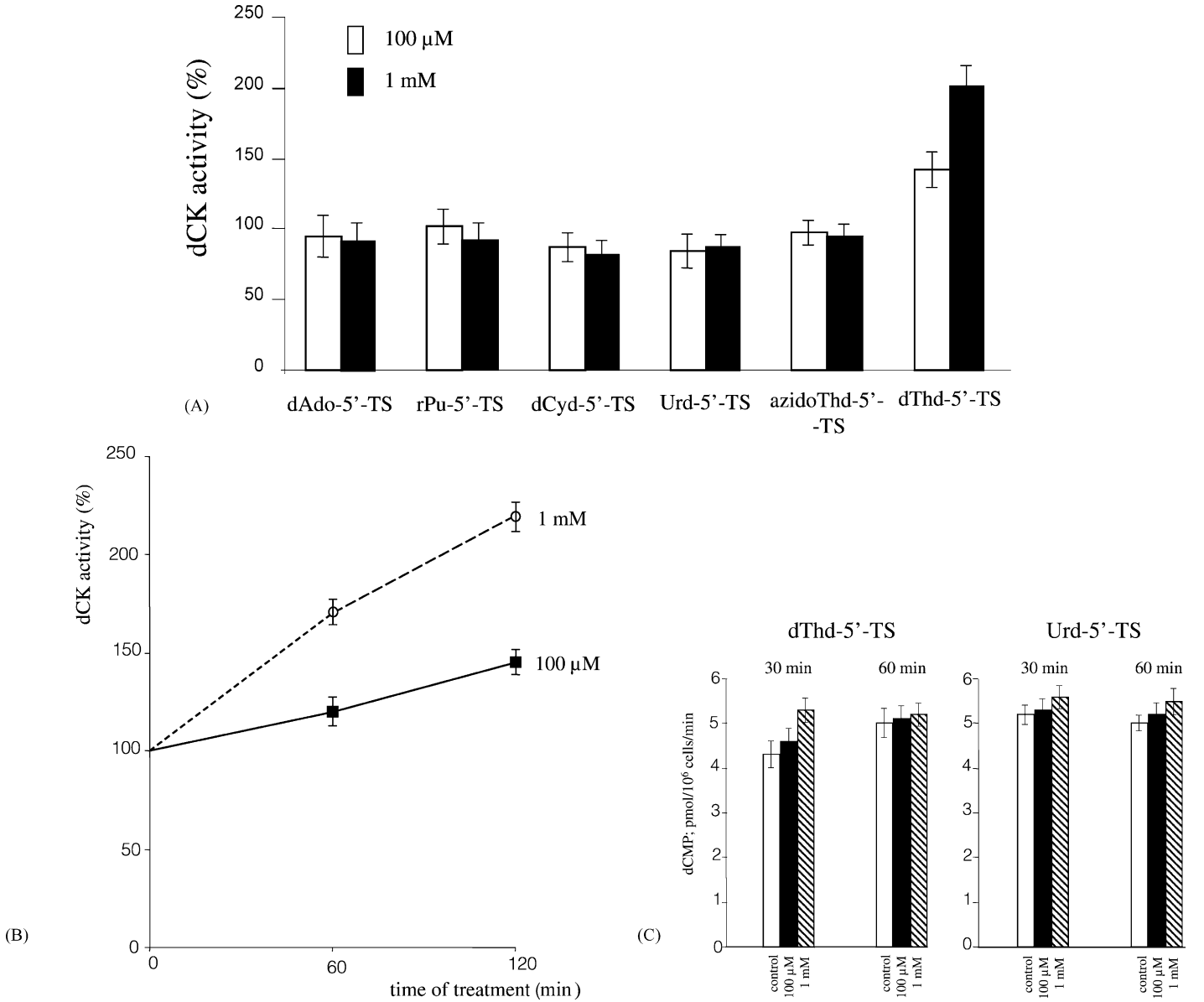

Fig. 1. Effect of different nucleoside-5′-TS treatments on dCK activity in primary human tonsillar lymphocyte cultures. (A) Parallel primary cultures of human tonsillar lymphocytes were incubated in the absence or presence of the drugs indicated at the bottom of the bar clusters (blank columns: 100 µM; filled columns: 1 mM) for 120 min at 37°, then washed twice, extracted, and dCK activity was determined in cell-free extracts with ^{3}H-dCyd substrate as described in Section 2. Activity values are expressed as percentages of the untreated control (control activity: 5.2 pmol/10^{6} cells/min). Error bars indicate the means of three parallels in a representative experiment, which was repeated at least three times. (B) Time- and concentration-dependence of dCK activation with dThd-5′-TS in human lymphocytes. (C) Effect of dThd-5′-TS and Urd-5′-TS on dCK activity in cell-free extracts. See Section 2 for details. dAdo, deoxyadenosine; rPu, ribopurine; dCyd, deoxycytidine; Urd, uridine; azidoThd, azidothymidine; and dThd, deoxythymidine.

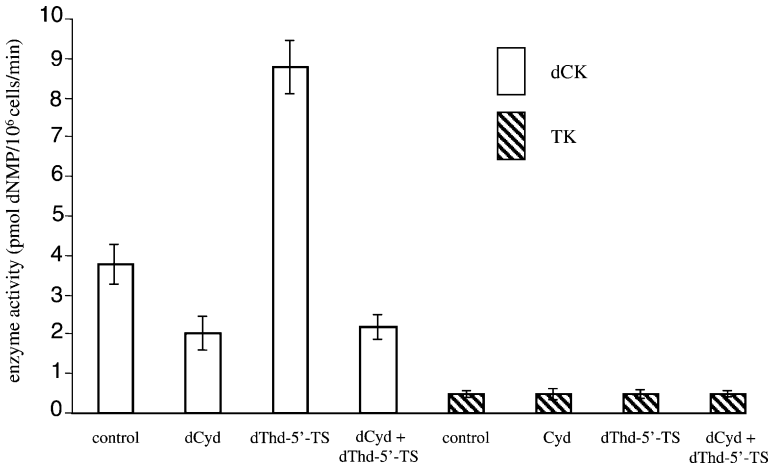

Fig. 2. Selective potentiation of dCK but not TK activities by dThd-5′-TS in primary PBMC cultures. Enzyme activity levels are shown in human PBMCs isolated from healthy human blood. Cells were treated with dThd-5′-TS alone (1 mM) or in combination with 100 μM dCyd for 120 min. dCK (blank bars) and TK (striped bars) enzyme activities were measured with ^{3}H-dCyd and ^{3}H-Thd, respectively, and expressed as picomoles of phosphorylated substrates, by the extracts of 10^{6} cells/min. TK2 activity did not change under conditions investigated.

on dCK activity (Fig. 3, second cluster of open bars). On the contrary, dCyd supplied at the same concentration could completely rescue the enzyme activation (Fig. 3, third cluster of open bars).

The concentration dependence of the dCyd counteracting effect has been also measured. Even 10 μM dCyd has proven to be as potent as 100 μM, but 1 μM was less effective (data not shown). Regardless of any completion with the natural deoxypyrimidines, TK activity shows a dose-dependent slight reduction in dThd-5′-TS-treated cells (striped bars).

3.3. The effect of dThd-5′-TS and Urd-5′-TS on the salvage of deoxypyrimidine nucleosides in tonsillar lymphocytes

To explore the possible impacts of dThd-5′-TS on the overall nucleotide salvage, measurements of labelled nucleotides in the pool and in DNA were performed. Primary lymphocyte cultures from tonsils were labelled with tritiated nucleosides in the presence of dThd-5′-TS or Urd-5′-TS for different time periods. The ethanol-soluble labelled pool (uptake) and the phosphorylated fraction (nucleotides) were measured as described in Section 2. In our terminology, the ‘‘uptake’’, fraction A, represents the pool of labelled nucleosides, nucleotides plus liponucleotides (dCDP-choline and dCDP-ethanolamine). The free nucleoside mono-, di- and triphosphates belong to the ‘‘phosphorylated’’ B cluster. As displayed, dThd-5′-TS but not Urd-5′-TS increases both the ‘‘uptake’’ (Fig. 4A) and the amount of ‘‘phosphorylated’’ (Fig. 4B) pool labelled with ^{3}H-dCyd, but decreases its incorporation into DNA to 50% of the control (Fig. 4C). The effect of dThd-5′-TS and Urd-5′-TS on the salvage of the pyrimidine counterpart was also measured, using ^{3}H-dThd as labelled precursor (Fig. 5). dThd-5′-TS elicited a severe depletion

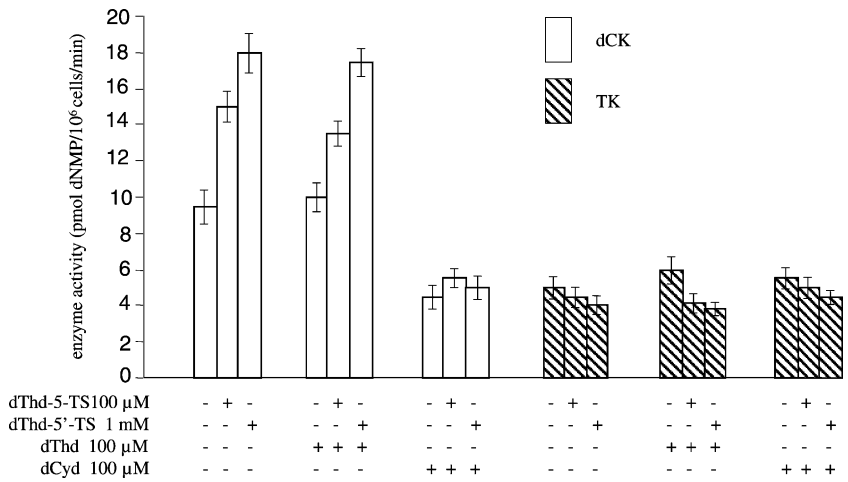

Fig. 3. dCyd but not dThd completely reverses the enhancing effect of dThd-5′-TS on dCK activity in treated cells. Primary tonsillar lymphocyte cultures were exposed to the indicated combinations of the thiosulphate analogue with the natural pyrimidine deoxynucleosides for 120 min at 37°. After the treatment, dCK and TK assays were simultaneously performed for 20 min with crude cell extracts, as described in Section 2.

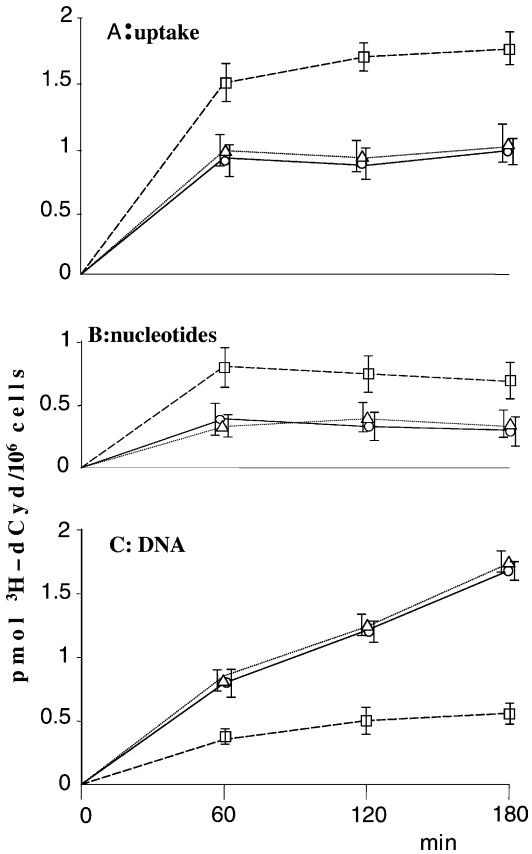

Fig. 4. The effect of dThd-5′-TS and Urd-5′-TS on ^{3}H-dCyd metabolism in lymphocytes. Cells were incubated in the absence (open circles) or in the presence of either 1 mM dThd-5′-TS (open squares) or 1 mM Urd-5′-TS (open triangles) for three different time periods, followed by pulse-labelling with 1.0 μCi/mL ^{3}H-dCyd for 20 min. Fractionation was performed as described in Section 2. Labelling was counted in ethanol-soluble (panel A, uptake), in DEAE–cellulose bound pool (panel B, phosphorylation or nucleotides) and in the ethanol-insoluble fractions (panel C, DNA incorporation) as described in Section 2. Data are mean values of three independent experiments; standard errors represented by vertical bars.

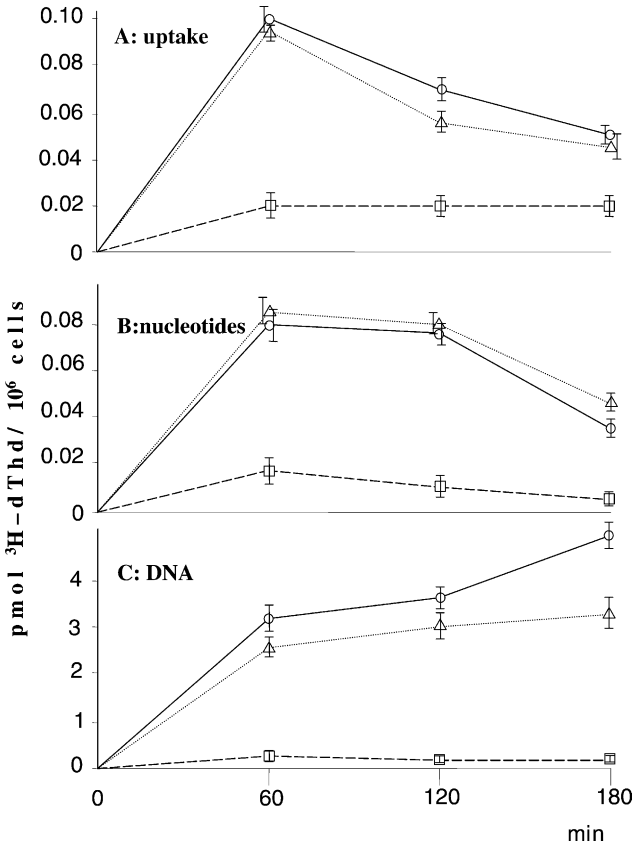

Fig. 5. The effect of dThd-5′-TS and Urd-5′-TS on ^{3}H-dThd metabolism in lymphocytes. Experimentation and the way of illustration are essentially identical with those applied in Fig. 4, except that ^{3}H-dThd (instead of ^{3}H-dCyd) was used for metabolic labelling. Note the different scale of the ordinate in panel C.

in each thymidine pool; especially the dwindled uptake, i.e. nucleoside pool (panel A) and phosphorylation, i.e. nucleotides (panel B) are diametrically the opposite of those seen with ^{3}H-dCyd labelling, while incorporation of dThd into DNA (panel C) is much more inhibited (by 90% of control, Fig. 5C) than that of dCyd (Fig. 4C) was.

4. Discussion

Here, we presented experiments concerning the effect of aliphatic thiosulphates (Bunte salts) – with antibacterial and fungicidal activity [27,28] – on the deoxynucleoside salvage processes and deoxynucleoside kinase activities in primary human lymphocyte cultures.

Lymphocytes were treated for 2 hr with 0.1 and 1.0 mM dAdo-, dCyd-, rPu-, riboUrd-, 3′-azido-Thd- and dThd-5′-TSs. Out of the six nucleoside-5′-TSs investigated, only the thymidine derivative, dThd-5′-TS, resulted in a significant (2- to 3-fold) dCK enzyme activation, already after 2-hr treatment, both in primary lymph node lymphocytes and in PBMCs (Figs. 1A and 2), while TK isoenzymes were not influenced under the same conditions.

The rate of phosphorylation of the dCK and TK substrates was not altered in the presence of either dThd-5′-TS or Urd-5′-TS at the same concentrations, in the cell-free extracts. This finding indicates that the increase in enzyme activity, measured after cell treatments, is not due to a simple interaction of the dCK protein with the dTMP thiosulphate analogue through a bipartite complex formation, but that it requires the context of intact intracellular environment, probably intact membranes, metabolic and/or regulatory pathways.

A tight connection between intact membranes and the phosphorylation process of dThd to dTTP was shown in tonsillar lymphocytes. Reversibly permeabilised cells phosphorylated labelled thymidine exclusively only to monophosphate (dTMP) [33]; no labelling could be detected either in dTDP–dTTP pools or in DNA. Intact cells converted dThd immediately up to dTTP and incorporated it into DNA. TK1 works in membrane damaged cells as well as in intact ones; however, the interconnection of TK to the next enzyme dTMP kinase was totally destroyed

in membrane damaged cells [33]. Not only the salvage of dThd requires intact plasma membranes, but as it has been shown, exogenous dCyd can be used with the same efficacy as (ribo)cytidine for membrane phospholipid synthesis in lymphocytes [10].

In quiescent lymphocytes (most of cells in tonsils and in PBMCs) the *de novo* nucleotide synthesis is of subsidiary importance, salvage networks being almost exclusively responsible for the proper maintenance of nucleotide pools [1,9,41]. The interconversion pathway between salvaged dCyd and dThd dominates in tonsillar lymphocytes, about 75% of salvaged dCyd is converted into dThd nucleotides, as has been shown [31]. To compensate the drop in thymidylate supply, dCMP produced by dCK can be transformed into dUMP by dCMP deaminase and subsequent methylation through thymidylate synthase yields dTMP, establishing a metabolic demand for dCK activation. The outlined interconversion pathway might still work even in the presence of excess dThd-5′-TS [32] serving the supplementation of thymidine nucleotides, while the transport and/or phosphorylation of dThd to dTTP in cells seems to be disturbed by the analogue.

The counteracting effect of dCyd but not that of dThd, preventing the activation of dCK by dThd-5′-TS, was surprising. Nevertheless, similar effects of dCyd were observed in totally different systems. Induction of the glycoprotein hormone α-subunit and placental alkaline phosphatase with butyrate was also inhibited by dCyd, and not by other nucleosides, in HeLa cells [36]. It has also been shown, that extracellular dCyd released the inhibitory effect of Cladribine on the dThd uptake and incorporation into DNA in human lymphocytes [31].

In this study it has been shown that a 5′-modified thymidine nucleoside monophosphate analogue elicits profound disturbances in cellular nucleoside metabolism and selectively potentiates dCK activity. These effects are very similar to those induced by widely used cytostatic and other genotoxic drugs. As dCK mRNA and protein expression levels have been found unchanged during short-term treatments, a post-translational covalent modification of the enzyme has been suggested [17]. During serial dilutions and affinity chromatography purification, the elevated enzyme activity survives, indicating, that feedback regulation by small metabolites or allosteric factors is less probable [18,21]. On the other hand, incubation with λ-protein phosphatase demolished the activated state, strongly referring to implication of reversible phosphorylation as putative means of enhancement [18]. It has also been described, that protein kinase C *in vitro* phosphorylates and ‘‘activates’’ the recombinant dCK [34], but these findings have not yet been confirmed under *in vivo* circumstances [35].

We assume, that the increase of dCK activity in cells is a compensatory mechanism, which might be induced by various signals, at different levels in the cell. Different drugs, irradiations can destroy integrity of membranes and metabolic pathways, inhibiting DNA synthesis and finally damaging it, which has to be repaired, or if it is no longer possible apoptosis will be induced. The elevated dCK activity will be able to supply cells with all dNTPs needed for the repair of DNA [41]. The counteracting effect of dCyd on the dThd-5′-TS treatment of cells might support the importance of the dCyd→dThd nucleotide interconversion pathway in regulatory processes too. In the present case the question emerges whether the activation of dCK should be ascribed to a signal coming from the perturbed nucleotide metabolic pathway, or from the damaged DNA in conjunction with its replication/repair apparatus. Recently, a new ribonucleotide reductase (RR), p53R2 was identified [37], and the level of the p53R2 was closely correlated to gamma-irradiation of cells, and plays a pivotal role in cell survival by repairing damaged DNA [38]. The change of mitochondrial permeability [39] and deficiency of TK isoenzymes and dCK, were also published [40] by long-term treatments with deoxynucleosite analogues.

To meet the needs for such different functions, a ‘‘DNA repair sensor’’ has to control short- and long-term adaptation functions via regulation of membrane processes, redoxy state of cells [43], nucleotide supply [42] and DNA repair. The molecular and cellular mechanisms, signals have to be identified by which changes in membranes, nucleotide metabolism and DNA synthesis/repair were observed, and transduced finally into upregulation of dCK activity.

Acknowledgments

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