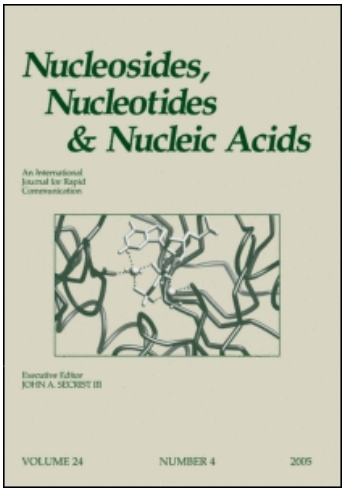

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M. Staub[a]; A. Stenger[b]; R. Sumeg[a]; T. Spasokoukotskaja[a]; L. D. Fairbanks[c]; H. A. Simmonds[c]; G. Keszler[a]
[a] Institute of Medical Chemistry, Molecular Biology and Pathobiochemistry, Semmelweis University, Budapest, Hungary [b] Health Service, Pediatric Department, Budapest, Hungary [c] Purine Research Unit, Guy's Hospital, London, United Kingdom

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THE NEUROLEPTIC CHLORPROMAZINE INHIBITS THE CATIONIC AND STIMULATES THE ANIONIC PHOSPHOLIPID PRECURSOR SYNTHESIS IN HUMAN LYMPHOCYTES

M. Staub □ *Institute of Medical Chemistry, Molecular Biology and Pathobiochemistry, Semmelweis University, Budapest, Hungary*

A. Stenger □ *Health Service, Pediatric Department, District II, Budapest, Hungary*

R. Sumeg and T. Spasokoukotskaja □ *Institute of Medical Chemistry, Molecular Biology and Pathobiochemistry, Semmelweis University, Budapest, Hungary*

L. D. Fairbanks and H. A. Simmonds □ *Purine Research Unit, Guy's Hospital, London, United Kingdom*

G. Keszler □ *Institute of Medical Chemistry, Molecular Biology and Pathobiochemistry, Semmelweis University, Budapest, Hungary*

□ *The widely used neuroleptic drug chlorpromazine (CPZ) influences membrane functions at the levels of ionic channels and receptors as shown. Here we show the effect of short term treatments by CPZ (30 μM), on the nucleotide-containing phospholipid precursors in human lymphocyte primary cultures. During 60 minutes incubation of the cells, the CDP-ethanolamine (CDP-EA) content was only slightly reduced (87 to 76 pmol/10^6 cells), the amount of CDP-choline (CDP-Ch) was inhibited totally (from 25 to 0 pmol) upon the treatment with 30 μM CPZ under the same conditions. It has been shown earlier, that dCTP can be used as well as CTP for biosynthesis of phospholipids. Thus, the separation of the corresponding ribo- and deoxyribo-liponucleotides was developed. CPZ almost completely inhibited the synthesis of both dCDP-EA and dCDP-Ch under the same conditions The synthesis of the activated liponucleotide precursors, can be measured by incorporation of extracellular 14C-dCyt into both dCDP-EA and dCDP-Ch, as shown earlier. While the cationic deoxyribo-liponucleotide content (dCDP-Ch, dCDP-EA) was decreased, the labelling of the anionic phospholipid precursor dCDP-diacylglycerol (dCDP-DAG) was enhanced several times, it could be labelled only in the presence of CPZ from 14C-dCyd. Thus, a principal disturbance of the membrane phospholipid synthesis is presented (i.e., inhibition of the cationic and enhancement*

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Address correspondence to M. Staub, Institute of Medical Chemistry, Molecular Biology and Pathobiochemistry, Semmelweis University, POB 260, H-1444, Budapest, Hungary. E-mail: staub@puskin.sote.hu

of the anionic dCDP-DAG synthesis). This profound influence on the membrane phospholipids by chlorpromazine, might be the primary effect that contributes to the wide spectrum of CPZ effects on neuronal cells.

Keywords Chlorpromazine; Liponucleotide; dCDP-choline; dCDP-ethanolamine; dCDP-DAG, metabolic labelling

INTRODUCTION

Chlorpromazine (CPZ), a cationic amphiphilic phenotiazine derivative, is the best known compound for the treatment of psychosis and schizophrenia.[1] Apart from its strong inhibitory effects on dopaminergic and serotoninergic neurotransmission[1], CPZ also has been shown to inhibit a number of ion channels and membrane pumps.[2,3] CPZ preferentially binds to neuron membranes known to be rich in anionic phospholipids, on account of its high affinity for the phosphate and carboxylic groups.[4]

Early studies indicated that CPZ modulates the cellular phospholipid biosynthesis probably via inhibition of phosphatidate phosphohydrolase,[5] as well as the turnover of CDP-DAG.

The influence of CPZ on the inositol phospholipid and nucleotide metabolism also has been investigated in human lymphocytes. Previously, we have shown an intensive salvage of deoxycytidine (dCyd) not only into DNA and dCTP, but also into a lipidic pool fraction of lymphocytes[6,7] and macrophages,[8,9] which were identified as phopholipid-nucleotide (liponucleotide) precursors. Deoxycytidine was incorporated into dCDP-ethanolamine and dCDP-choline even more effectively than ribocytidine (rCyd).[7] The inositol-phospholipid pathway has a fast turnover; its activated precursor is CDP-diacylglycerol (CDP-DAG). This pathway also could be labelled from exogenous dCyd, but only in the presence of CPZ and dCDP-DAG as it was identified in different cells.[6–9]

The aim of the present study was to investigate the effects of chlorpromazine (CPZ) on membrane ribo- and deoxyribo-phospholipid precursor concentrations and their biosynthesis in human lymphocytes.

MATERIALS AND METHODS

Isolation of human tonsillar lymphocytes was performed essentially as described previously.[6,7] Primary cell cultures (10^7 cells/ml) were maintained in the presence or in the absence of CPZ (Sigma-Aldrich, St. Louis, MO, USA) in Eagle's MEM, for the indicated times. Finally, metabolic processes were stopped by 1.0 ml 10% TCA. Before HPLC analysis, TCA was

extracted with diethylether to achieve pH 6-7 according to the procedure developed in Simmonds et al.[10]

Labelling with ^{14}C-deoxycytidine: Cell cultures were labelled with ^{14}C-dCyd (10^7 cells/ml/0,2 μCi, spec. act.:16 Ci/mmol, Amersham) at 37°C in Eagle's MEM in the presence or absence of the drug, as indicated. At the end of the incubation, cells were washed twice with ice-cold PBS and centrifuged for 2 minutes at 3,300 rpm.

The cell pellet was resuspended into 250 μl distilled water and 250 μl of 10% TCA, while mixing vigorously on a vortex. The mixture was centrifuged for 2 minutes at 5,000 rpm. The TCA in the supernatant was back-extracted with water-saturated diethyl ether until the pH of aqueous layer was higher than pH 5. The extract was analyzed immediately by injecting 100 μl onto anionic Phenomenex column by a Merck HPLC system with a diode array detector as described in Simmonds et al.[10] All chemicals used for HPLC separations were analytically pure and were purchased from Sigma.

RESULTS AND DISCUSSION

Freshly isolated human lymphocytes were incubated for 0, 30, and 60 minutes with and without 30 μM CPZ. After treatment, cells were extracted with TCA and the ribo- and deoxyribo-liponucleotides (CDP-ethanolamine CDP-EA, dCDP-EA) and CDP-choline (CDP-Ch, dCDP-Ch) were separated from other nucleotide-conjugated metabolites according to the HPLC method developed in Ref. 10. As it can be seen in Table 1, 10^6 human tonsillar lymphocytes contained 84–87 pmol CDP-EA and only 21–25 pmol CDP-Ch. These values did not change significantly within a 60 minutes incubation at 37°C (control 0, 30 and 60 minutes, respectively). Treatment of cells with 30 μM chlorpromazine slightly decreased the cellular CDP-EA content, however the CDP-Ch levels dropped to zero, an undetectably low concentration following either a 30 minutes or 60 minutes incubation with CPZ, supporting a more sensitive process of CDP-Ch synthesis. Arabinosyl-cytosine (araC) was also tested at 50 nM that is known to effectively inhibit DNA synthesis. It slightly increased the CDP-choline pool but the CDP-EA pool was not affected (Table 1).

Previously we have shown that deoxycytidine (dCyt) is not only incorporated into DNA but also into lipidic compounds in lymphocytes[6,7] and also in macrophages.[8] It was found, that dCyd was incorporated into dCDP-EA and dCDP-Ch as effectively as ribocytidine (Cyd). The fast-turnover inositol-phospholipid pathway, dCDP-diacylglycerol (dCDP-DAG) also could be labelled from external dCyd, but only in the presence of CPZ.[6–9] The DNA synthesis inhibitor araC increased the amount of the deoxyribo-liponucleotides (Table 1) parallel to inreasing the dCTP pool too.[7]

TABLE 1 The Concentration of Ribo-Liponucleotides (CDP-EA/CDP-Choline) and the Labelling of Deoxyribo-Liponucleotides (dCDP-EA/dCDP-Choline) in Control, CPZ- and Ara-C-Treated Human Lymphocytes

	Control			CPZ		araC	
	0 min	30 min	60 min	30 min	60 min	30 min	60 min
	Pmol/10^6 cells						
CDP-choline	25.3 ± 1.8	24.6 ± 2.1	21.2 ± 1.2	0.0	0.0	31.7 ± 1.9	33.5 ± 2.3
CDP-EA	87.6 ± 6.6	84.0 ± 7.2	85.2 ± 5.9	76.6 ± 5.5	76.2 ± 7.8	88.6 ± 9.1	85.3 ± 7.9
	^{14}C-dCyd incorporated (% of total uptake)						
^{14}C-dCDP-choline	12.0 ± 0.8	10.9 ± 0.9	9.3 ± 0.8	0.0	0.0	10.0 ± 1.1	25.3 ± 1.6
^{14}C-dCDP-EA	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.0	0.0	0.6 ± 0.1	14.8 ± 0.1

Cells were cultured in Eagle-MEM in the absence (control) or in the presence of CPZ (30 μM) or ara-C (50 nM) for indicated times. The ribo-liponucleotides (CDP-Ch, CDP-EA) and deoxyribo-liponucleotides (dCDP-Ch, dCDP-EA) were separated by HPLC and the concentrations were determined according;[10] data are expressed as pmol liponucleotide/10^6 cells.

Deoxyribo-liponucleotides were determined after cell labelling with ^{14}C-dCyd. The acid soluble pool was separated and radioactivity was determined in fractions dCDP-choline/dCDP-EA as described in.[10] Data are expressed as percentage of total uptake radioactivity.

Data are means of 3 independent experiments; standard deviations are indicated. The changes of dCDP-Ch and dCDP-EA concentration and labelling are highly significant.

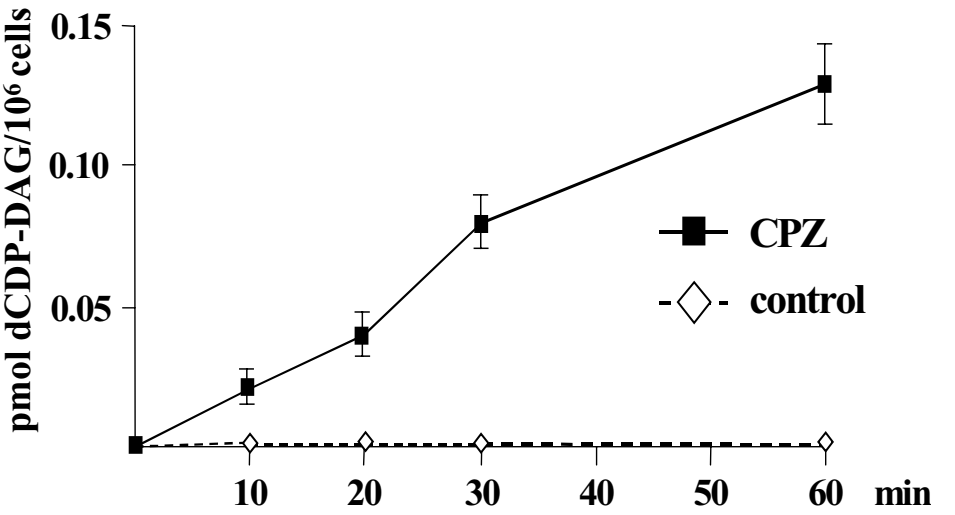

FIGURE 1 Effect of CPZ on the dCDP-DAG synthesis in lymphocytes as followed by ^{14}C-dCyd incorporation. Cells (10^7/ml) were incubated with 0.2 μCi ^{14}C-dCyd, in the absence (dashed line) or in the presence of 30 μM CPZ (solid line) for indicated times. Cells were washed, extracted and the amount of ^{14}C-dCDP-DAG was determined after separation on HPLC as described.[10]

The amount of the deoxyribo-liponucleotides (dCDP-Ch and dCDP-EA) was also measured after separation by HPLC. It turned out that the synthesis of dCDP-Ch and dCDP-EA is even more sensitive to CPZ, than that of the corresponding ribolipo-nucleotides. No absorbing compounds were found in the fractions corresponding to the deoxyribo-liponucleotides (dCDP-Ch and dCDP-EA) after incubation of lymphocytes with CPZ (Table 1).

The biosynthetic precursor for the anionic inositol-phospholipids, phosphatidyl-glycerol, and cardiolipine, is CDP-diacylglycerol (CDP-DAG), a lipid-soluble compound. We previously showed that exogenous deoxycytidine can also be incorporated into this pool, revealing the existence of dCDP-DAG in cells.[6–9] Here, we determined the amount of ^{14}C-dCDP-DAG in control as well as in chlorpromazine-treated lymphocytes (Figure 1). This compound was practically not labelled in control cells (dashed line). In contrast, CPZ treatment resulted in a time-dependent, dramatic expansion of the dCDP-DAG labelling from extracellular dCyd (solid line, Figure 1).

The enhancement of dCDP-DAG synthesis by chlorpromazine might be due to the inhibition of phosphatidate phosphatase and the consequent accumulation of phosphatidic acid.[5] Chlorpromazine also blocks the activity of CTP:phosphocholine cytidylyltransferase[11] that might explain the low levels of liponucleotides in cells treated with chlorpromazine (Table 1). Importantly, the labelling of the dUMP and dTTP pools from extracellular deoxycytidine is significantly increased in the presence of chlorpromazine (data not shown). This observation clearly indicates that upon inhibition

of liponucleotide synthesis, a greater part of labelled deoxycytidine is converted to thymine nucleotides by the concerted action of dCMP deaminase and thymidylate synthase (known as deoxypyrimidine interconversion pathway). In a broader sense, these results point to a tight relationship between the nucleotide and membrane phospholipid metabolism in cells.

The role of rCTP in membrane phospholipid biosynthesis was discovered by Kennedy et al.,[12] who showed that the relevant cytidylyltransferase could use not only rCTP but also dCTP. However the function of deoxyliponucleotides is still unknown. It has been shown that they are synthesised from a separate compartment of dCTP that is distinguishable from the de novo dCTP pool.[13] Two separate dCTP pools exist also in tosillar lymphocytes—one for DNA replication, the other for DNA repair and liponucleotide pools.[6]

An uninterrupted supply of ribo- and deoxyribonucletides is essential for membrane precursor-, and RNA and DNA synthesis in mitogen-stimulated T lymphocytes.[14] Therefore, membrane precursor and DNA synthesis seem to be tightly coupled. The specific role of dCyd in membrane synthesis is supported by results showing that rCyd was not able to replace dCyd as precursor for liponucleotide synthesis.[7]

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