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# Cytidine triphosphate synthetase (CTP synthetase) as a druggable target in cancer

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**Abstract**

Cytidine triphosphate (CTP) synthetase is a key enzyme in the biosynthesis of pyrimidine ribonucleotides. The enzyme catalyzes the conversion of uridine triphosphate (UTP) to CTP and is the predominant pathway for the synthesis of CTP in proliferating and malignant tissues. Elevated CTP synthetase activity is seen in various malignancies, such as acute lymphocytic leukemia (ALL), acute myeloid leukemia (AML), hepatoma, colon cancer and renal carcinoma, as well as non-Hodgkin's lymphoma (NHL). The high activity of the enzyme has led to the development of inhibitors as potential new therapeutic tools. The best explored inhibitor of CTP synthetase is cyclopentenyl cytosine (CPEC), which has been investigated in both preclinical and clinical studies. CPEC proved to profoundly inhibit CTP synthetase *in vitro* in colon carcinoma, ALL and AML cell lines. Moreover, CPEC has been shown to inhibit the growth of human and murine leukemia xenografts *in vivo*. A phase I clinical trial with CPEC as monotherapy showed that this compound inhibits CTP synthetase in surrogate tissues such as bone marrow. However, at the highest doses, CPEC proved to have severe cardiovascular toxicity, the mechanism of which has not been unraveled until recently. Additional preclinical studies did not show any cardiotoxicity in rats. There is probably a good rationale for using CPEC at lower doses, since it has been shown in preclinical models to display synergistic cytotoxic effects with other nucleoside analogues such as cytarabine or gemcitabine. In conclusion, CTP synthetase is a druggable target, especially for combination treatment of AML and ALL.

## Introduction

This review describes several aspects concerning the synthesis of pyrimidine (deoxy)ribonucleotides in cancer cells, and more specifically in leukemic cells. Previous studies have shown alterations in ribonucleotide concentrations in various malignancies, including hematological malignancies (1-4). In most of these studies, an increase in ribonucleotide concentrations was observed in the tumor cells compared with their nonmalignant counterparts. This increase concerned predominantly concentrations of the pyrimidine ribonucleotides cytidine triphosphate (CTP) and uridine triphosphate (UTP), particularly the concentrations of CTP in tumor cells compared with nonmalignant cells (1-4).

The three possible metabolic pathways for the synthesis of CTP are extensively described later in this review. Briefly, CTP can be synthesized via the salvage pathway of uridine and the pyrimidine ribonucleotide *de novo* synthesis pathway on the one hand, both culminating in the conversion of UTP to CTP, catalyzed by the enzyme CTP synthetase (Fig. 1). On the other hand, CTP can also be formed by the salvage pathway of cytidine. Previous studies have provided evidence of a high metabolic flux through the uridine salvage pathway in leukemic cells (5) and solid tumors (6).

The fact that the rate-limiting step in the formation of CTP through this salvage pathway of uridine is catalyzed by CTP synthetase, together with evidence for high mean concentrations of CTP in cancer cells (3), indicates that CTP synthetase is a potential target in cancer cells. This target could provide a new chemotherapeutic approach using specific inhibitors of CTP synthetase, such as cyclopentenyl cytosine (CPEC). Inhibition of CTP synthetase would lead to decreased synthesis of CTP (7-12) and deoxyCTP (dCTP) (9, 10) in cancer cells, resulting in decreased RNA and DNA synthesis (7, 9, 12), and therefore a cytostatic and/or cytotoxic effect. Moreover, decreased concentrations of dCTP might increase the cytotoxic effects of nucleoside analogues such as cytarabine and gemcitabine in cancer cells (13-15). The interference of dCTP with cytarabine and gemcitabine metabolism will be described later on in this review.

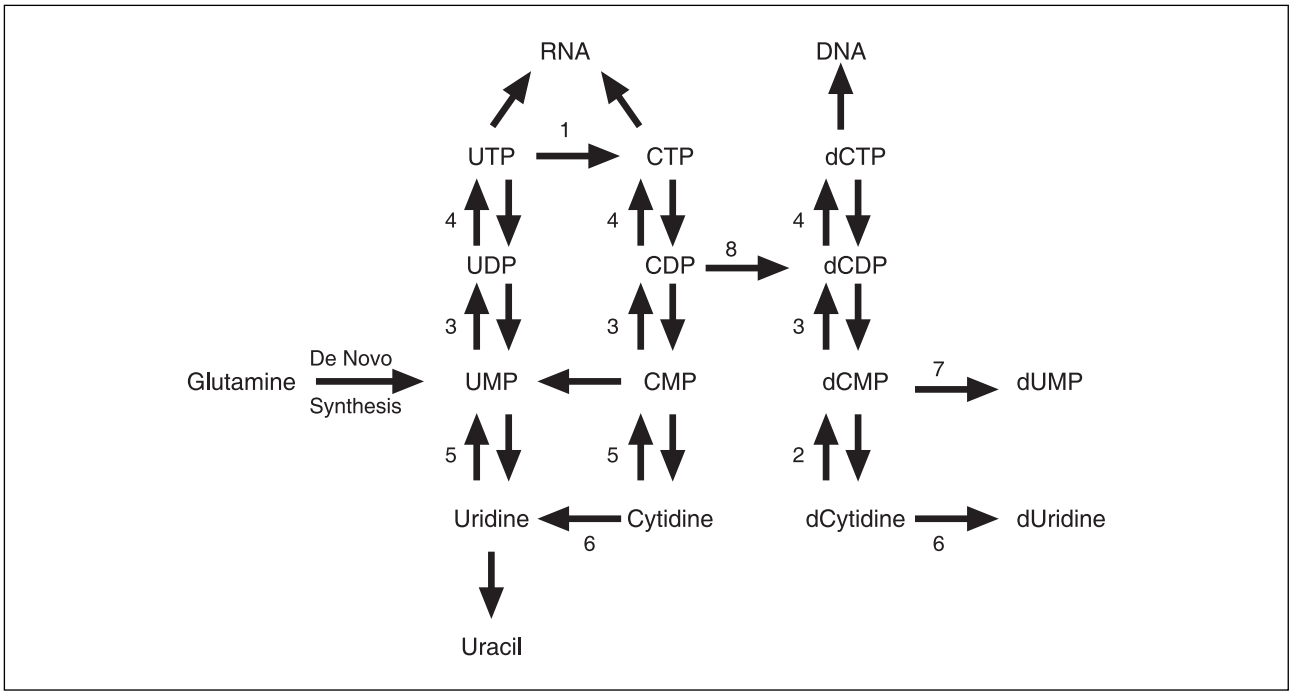


Fig. 1. Scheme representing the pyrimidine *de novo* synthesis and salvage pathways. Glutamine can be metabolized via several steps to uridine monophosphate (UMP), which in turn can be phosphorylated to uridine diphosphate (UDP) and uridine triphosphate (UTP). CTP synthetase (1) catalyzes the conversion of UTP to cytidine triphosphate (CTP). The middle trunk represents the salvage pathway of cytidine and the right trunk represents the deoxycytidine salvage pathway, where deoxycytidine is phosphorylated to deoxyCTP (dCTP) by three consecutive steps catalyzed by deoxycytidine kinase (dCK) (2), nucleoside-phosphate kinase (NMP-kinase) (3) and nucleoside-diphosphate kinase (NDP-kinase) (4). (5): uridine/cytidine kinase. The conversion of deoxycytidine to deoxyuridine (6) is catalyzed by deoxycytidine deaminase. dCMP deaminase (7) converts deoxyCMP (dCMP) to deoxyUMP (dUMP). (8): ribonucleotide reductase.

## Synthesis of ribonucleotides and deoxyribonucleotides

### *General introduction*

Ribonucleotides, or ribonucleoside phosphates, are molecules consisting of one of the bases uracil, cytosine, adenine or guanine covalently bound to a ribose molecule and one, two or three phosphate ions. One can distinguish nucleoside monophosphates (NMP), nucleoside diphosphates (NDP) and nucleoside triphosphates (NTP), which are interconvertible by virtue of the phosphorylating enzymes NMP-kinase and NDP-kinase and by (dephosphorylating) phosphatases. Ribonucleotides are classified into purine ribonucleoside phosphates on the one hand, consisting of the phosphorylated forms of the nucleosides adenosine and guanosine. On the other hand, the pyrimidine ribonucleoside phosphates consist of the phosphorylated nucleosides cytidine and uridine.

Ribonucleotides are essential elements for cellular homeostasis as they are precursors for RNA and DNA. Some nucleotides (guanosine triphosphate [GTP], adenosine 3',5'-cyclic monophosphate [cAMP]) are involved in signal transduction pathways and represent an essential source of energy (adenosine triphosphate [ATP]). Moreover, ribonucleotides such as ATP, GTP and UTP may supply a phosphate ion to other molecules and some derivatives of nucleotides (CDP-choline, CDP-ethanolamine, CDP-diacylglycerol) are precursors of phospholipids (16) and therefore play a role in the biosynthesis of cell membranes. The intracellular concentrations of the various purine and pyrimidine ribonucleotides differ and depend on several aspects of the cell, *i.e.,* cell type, proliferation and degree of differentiation. The contribution of these three aspects to the concentrations of the various ribonucleotides in most tissues can be summarized as follows:

- In nonproliferating tissues, a predominance of purine ribonucleotides is observed, consisting mainly of adenosine nucleotides (ATP). In addition, CTP is the ribonucleotide with the lowest concentration (1, 6, 17), leading to high UTP/CTP and purine/pyrimidine ratios. The pattern of ribonucleotide concentrations in differentiated cells shows a predominance of purine ribonucleotides and low concentrations of CTP.
- In proliferating tissues (whether or not of malignant origin), the absolute concentration of CTP is increased compared to in nonproliferating cells. Furthermore, the relative amount of CTP is increased compared to other ribonucleotides (1-3, 6, 18, 19). Concomitantly, the concentration of ATP is decreased, leading to lower

purine/pyrimidine and UTP/CTP ratios in proliferating cells as compared with nonproliferating cells.

One could therefore hypothesize that the ribonucleotide showing the lowest concentration in nonproliferating cells (CTP) is more abundantly present in proliferating cells and is more readily available for the metabolic processes that are required for cell proliferation.

*Deoxynucleotides*

Deoxynucleotides can be synthesized via either phosphorylation of the various deoxynucleosides or by reduction of the ribonucleoside diphosphates catalyzed by the enzyme ribonucleotide reductase. Subsequently, the deoxyribonucleoside diphosphates can be phosphorylated to deoxyribonucleoside triphosphates (dNTP) and may be incorporated into DNA. Ribonucleotide reductase has a general catalytic site and substrate-specific sites and is capable of reducing adenosine diphosphate (ADP), guanine diphosphate (GDP), cytidine diphosphate (CDP) and uridine diphosphate (UDP) (16). Therefore, a direct relationship exists between ribonucleotides and deoxyribonucleotides, and alterations in the concentrations of the former will influence the synthesis of the latter (20).

In proliferating and/or malignant tissues, alterations in deoxyNTP (dNTP) concentrations have been observed. In rat hepatoma, increased concentrations of dNTP were detected and a correlation was seen between the rate of proliferation and the increase in dNTP concentrations (1). The major increase was observed in dCTP concentrations. In conclusion, similar to the observations concerning ribonucleotide concentrations in proliferating cells, the balance of deoxyribonucleotides is shifted towards the pyrimidine deoxyribonucleotides, mainly towards the synthesis of dCTP.

*CTP synthesis as part of pyrimidine ribonucleotide synthesis*

Three major pathways contribute to the synthesis of pyrimidine ribonucleotides and several links exist among these pathways (Fig. 1): 1) the pyrimidine *de novo* pathway; 2) the salvage pathway of uridine; and 3) the salvage pathway of cytidine.

1. The pyrimidine *de novo* pathway

This pathway constitutes the formation of uridine monophosphate (UMP) via several steps from glutamine (21). The first step is characterized by the formation of carbamoyl phosphate from glutamine and $HCO_3^-$, catalyzed by *carbamoyl phosphate synthetase type II*. The subsequent step yields the formation of *N*-carbamoyl aspartate and is catalyzed by *aspartate carbamoyltransferase*. This enzyme is feedback-inhibited by CTP (22). In the subsequent step, the pyrimidine ring is formed, yielding dihydroorotate (catalyzed by *dihydroorotase*), which is transported into the mitochondrion and dehydrogenated to orotate by the enzyme *dihydroorotate dehydrogenase*. Subsequently, orotate is transported from the mitochondrion to the cytosol and acquires a phosphoribosyl moiety in the next step catalyzed by *orotate phosphoribosyltransferase,* forming orotidine monophosphate (OMP). The final step in the formation of UMP consists of the decarboxylation of OMP, catalyzed by *OMP decarboxylase*.

2. The salvage pathway of uridine

This pathway results in the formation of UTP from the nucleoside uridine. Uridine can be phosphorylated to UMP by the enzyme uridine kinase, which is considered to be the rate-limiting step in the pyrimidine salvage pathways (23). The subsequent phosphorylating steps are catalyzed by the enzymes NMP-kinase (forming UDP) and NDP-kinase (forming UTP) (Fig. 1).

UMP synthesized via the pyrimidine *de novo* pathway as decribed previously is also phosphorylated to UTP by the subsequent catalytic actions of NMP-kinase and NDP-kinase.

3. The salvage pathway of cytidine

This pathway results in the phosphorylation of cytidine via three steps, culminating in the formation of CTP. The three steps are catalyzed by the same enzymes as described for the salvage pathway of uridine (Fig. 1). Although the enzymes of the cytidine salvage pathway are similar to those of the uridine salvage pathway, the affinities of each enzyme for their respective substrates may vary, depending on the type of tissue and the state of (malignant) proliferation.

4. Links between the salvage pathways of uridine and cytidine

Both pathways are interconnected by three enzymes. Cytidine deaminase converts cytidine to uridine, while dCMP deaminase catalyzes the conversion of deoxyCMP (dCMP) to deoxyUMP (dUMP). The most important link between the salvage pathways of uridine and cytidine is the enzyme CTP synthetase, which catalyzes the conversion of UTP to CTP. This enzyme is crucial for the cell, since it supplies a second pathway for the synthesis of CTP. The enzyme CTP synthetase will be extensively discussed further on in this review.

The different affinities of the enzymes involved in the salvage pathways of uridine and cytidine for the various substrates originating from uridine or cytidine in different tissues often result in the preference of each tissue to use predominantly either the uridine or the cytidine salvage pathways for the synthesis of pyrimidine ribonucleotides. This may have clinical relevance, since the inhibition of either of the pathways may have a tissue-selective effect, resulting in reduced synthesis of pyrimidine ribonucleotides.

*CTP synthetase*

1. Enzymatic properties

The enzymatic properties of CTP synthetase (EC 6.3.4.2, CTP synthase, UTP—ammonia ligase) were

described as early as 1956 (24). The enzyme catalyzes the ATP-dependent transfer of an amide nitrogen from glutamine or $NH_3$ to the C4 position of UTP to form CTP.

CTP synthetase has been isolated and/or purified from *Escherichia coli* (25), *Saccharomyces cerevisiae* (26), bovine liver (27, 28), rat liver (29, 30) and Ehrlich ascites cells (31). The enzyme may be present in a monomer, dimer or tetramer configuration, with a predominance of tetramers in the presence of saturating concentrations of UTP and ATP (25, 32). The tetramer of CTP synthetase proved to have higher specific activity compared with the dimer and monomer configurations (30, 33). The molecular weight of the CTP synthetase monomer in *E. coli* is 60,438 Da (34), which is slightly less than the molecular weight of 66 kDa observed for the enzyme purified from mammalian liver tissue (30).

There is convincing evidence that a second isoform of CTP synthetase may be present in malignant tissues (31), as has been described in *S. cerevisiae* (35, 36). In our laboratory, cDNA coding for a second isoform of CTP synthetase has been isolated (37). The nucleotide sequence showed 67% homology with the previously cloned human CTP synthetase, while the predicted amino acid sequence presented 74% homology. Expression of this CTP synthetase cDNA in an *E. coli* strain lacking endogenous CTP synthetase activity and thus requiring cytidine for its growth showed that the bacteria could grow in the absence of cytidine (37).

CTP synthetase is inhibited by CTP in yeast (26) and mammalian cells (38), and this feedback inhibition plays an important role in the balance of pyrimidine ribonucleotides. Some mutant cell lines lacking this inhibitory effect of CTP possess high intracellular concentrations of CTP and dCTP, resulting in an increased rate of spontaneous mutations induced by the imbalance of deoxyribonucleotides (20, and resulting in resistance to deoxynucleoside analogues (39).

## 2. Molecular biological aspects

CTP synthetase of *E. coli* (34) and *S. cerevisiae* (35) has been cloned, as well as the cDNAs of the two isoforms of the human enzyme (37, 40). The two human CTP synthetase genes are located on 1p34.1 and Xp22 (37, 41). Mutations in the human CTP synthetase gene may result in different kinetic properties for this enzyme, such as diminished CTP inhibition, leading to higher concentrations of CTP and therefore resistance to nucleoside analogues such as cytarabine (araC) (42).

## 3. Activity of CTP synthetase in nonmalignant tissues

The activity of CTP synthetase has been studied in various nonmalignant rat tissues, with high activity observed in testis (43), thymus and spleen tissue (44), moderate activity in brain, kidney, adipose, bone marrow and lung tissue (43, 44), and low to moderate activity in blood, lymph node, heart, liver, skeletal muscle and gut tissue (43-46). In our laboratory, we investigated the activity of CTP synthetase in various human blood cells isolated from healthy volunteers by density centrifugation and elutriation centrifugation. The highest CTP synthetase activity was found in thrombocytes, followed by monocytes and lymphocytes, with rather low activity in granulocytes and erythrocytes (47). Furthermore, the activity of CTP synthetase was investigated in $CD34^+$ bone marrow cells, representing the nonmalignant counterpart of leukemic cells at the same stage of differentiation. The activity in nonmalignant bone marrow cells appeared to be slightly, although not significantly, lower compared to malignant lymphoblasts (48).

Apparently, some tissues with a high proliferation rate (testis, spleen, thymus, bone marrow) present high CTP synthetase activity in order to fulfill their metabolic requirements. In regenerating and therefore proliferating rat liver tissue, modestly increased activity of CTP synthetase was observed as compared with normal liver tissue (29, 44). On the contrary, low activity was found in colon mucosa, which is considered a tissue that is repetitively renewing and therefore dividing (44, 45).

## 4. Activity of CTP synthetase in malignant tissues

The presence of CTP synthetase in a malignant hepatoma cell line was described as early as 1960 (49). Extensive studies on the role of CTP synthetase in pyrimidine nucleotide metabolism in malignancies were performed in the 1970s and 1980s by Weber and co-workers (6, 19, 29, 44, 45). High CTP synthetase activity was observed in several malignancies concomitantly with increased activities of several other enzymes of the pyrimidine *de novo* or salvage pathways (6, 19, 29, 45). In rat hepatomas, a correlation was observed between the doubling time of the tumors and CTP synthetase activity, and the enzyme is therefore considered to be the rate-limiting enzyme for the synthesis of CTP in rat hepatomas (19, 44). CTP synthetase activity was higher in hepatomas with a similar proliferation rate as regenerating rat liver tissue (44). These observations indicate that high CTP synthetase activity is not solely due to the proliferative state of hepatomas and is not related to the immaturity of the tissue, but rather appears to be related to the process of malignant transformation (19, 29).

High CTP synthetase activity was also observed in Ehrlich ascites tumor cells (43), myeloma (43), rhabdomyosarcoma (6, 43) and renal cell carcinoma cells of rats and humans (44). In a pheochromocytoma cell line, high CTP synthetase activity was suspected after studying the fluxes of radiolabeled nucleosides (50), showing that a considerable proportion of radiolabeled uridine was incorporated into CTP. The presence of high CTP synthetase activity in neuroblastoma cells was confirmed by the observation that a specific inhibitor of CTP synthetase could strongly deplete the CTP pools in neuroblastoma cell lines (51).

In hematological malignancies, high CTP synthetase activity was suggested by the high concentrations of CTP that were observed in myeloblasts and lymphoblasts (2, 3). Moreover, by studying fluxes of radiolabeled uridine and cytidine in a MOLT-3 T-lymphoblastic cell line, it was demonstrated that the majority of CTP is synthesized via

the salvage pathway of uridine and thus via CTP synthetase (5). High levels of CTP synthetase activity were observed in samples from adults with acute lymphocytic leukemia (ALL) and in some subtypes of non-Hodgkin's lymphoma (NHL) compared to nonmalignant lymphoid tissue (46). In chronic lymphocytic leukemia (CLL), the enzyme activity was not or only moderately higher (46) than in control tissue. In an HL-60 myeloid leukemia cell line, an essential role for CTP synthetase could be deduced from the observation that treating the cells with inhibitors of CTP synthetase depleted CTP pools and induced growth inhibition (7, 52).

In our studies on the *in vitro* activity of CTP synthetase in leukemic samples from 57 pediatric patients suffering from ALL, significantly higher activity of CTP synthetase was detected in ALL cells (6.5 ± 3.9 nmol CTP/mg/h) compared to lymphocytes from healthy controls (1.8 ± 0.9 nmol CTP/mg/h), which was independent of white blood cell count (WBC), blast percentage, age, gender or type of ALL (48). Enzyme activity was also not correlated with CTP synthetase mRNA expression (48). With respect to pediatric acute myeloid leukemia (AML), CTP synthetase activity in leukemic cells (5.1 ± 2.3 nmol CTP/mg/h) was significantly higher compared with granulocytes from healthy controls (0.6 ± 0.4 nmol CTP/mg/h), but was not different from CTP synthetase activity in nonmalignant $CD34^+$ bone marrow cells (5.6 ± 2.4 nmol CTP/mg/h) (53).

## Intervening in nucleotide synthesis for therapeutic purposes

### *Inhibition of CTP synthetase*

The predominant role of CTP synthetase in malignant tissues has been a strong argument in favor of developing inhibitors of this enzyme that would have specific antitumor effects with less toxicity for nonmalignant tissues. In the 1960s and 1970s, 3-deazauridine (DAU) was investigated as a competitive inhibitor of CTP synthetase. DAU proved to have a CTP-depleting effect in various solid tumor cell lines (54) and hematological malignancies (55), and decreased RNA and DNA synthesis (54) in tumor cell lines. However, the clinical application of DAU was disappointing, showing little therapeutic efficacy in clinical trials in patients with colon carcinoma (56) and hematological malignancies (57).

### *Cyclopentenyl cytosine (CPEC)*

1. Chemistry and intracellular metabolism

In the 1980s, CPEC (NSC-375575) was developed as a novel cytidine analogue (58, 59) in which the furan ring of the ribose sugar was replaced by a cyclopentenyl moiety (Fig. 2).

The transmembrane transport of CPEC occurs predominantly by facilitated transport (9), which can be inhibited by uridine and cytidine (9). Subsequently, CPEC is phosphorylated by the enzyme uridine/cytidine kinase (9, 11, 59). CPEC monophosphate (CPEC-MP) is subsequently phosphorylated by NMP-kinase and CPEC diphosphate (CPEC-DP) by NDP-kinase to form CPEC triphosphate (CPEC-TP). CPEC-TP is the major metabolite of CPEC in myeloid and lymphocytic leukemia cell lines (7, 9, 12), as well as in samples from leukemia patients (48, 53) and colon carcinoma cell lines (60), with 100-fold higher intracellular concentrations of CPEC-TP compared to extracellular concentrations of CPEC (9, 12). Moreover, in colon carcinoma and leukemia cell lines, the amount of deaminated CPEC phosphates (CPEU-MP/-DP/-TP) is < 3% of all the phosphorylated CPEC compounds (9, 12, 60).

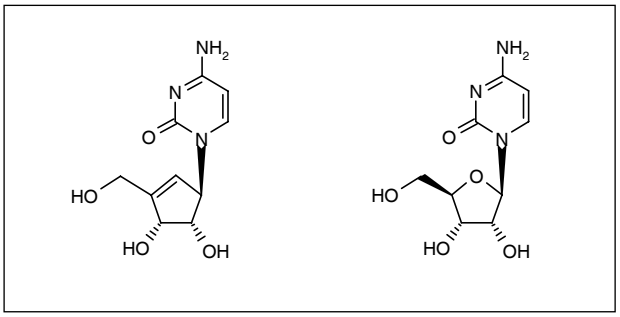

Fig. 2. Structural formula of CPEC (left) and cytidine (right).

CPEC-TP is the only metabolite of CPEC that has the ability (strong) to inhibit CTP synthetase at low (submicromolar) concentrations (12). In contrast, only very high concentrations of CPEC-MP and CPEC-DP (325-650 μM) inhibit CTP synthetase (12). CPEC-TP proved to be a noncompetitive inhibitor of CTP synthetase (11, 12). After removal of the drug from the cells, the inhibitory effect remains for 24-96 h, depending on the cell type, incubation time and concentration (7, 9, 12, 59-61). The half-life of CPEC-TP in a MOLT-4 lymphoblastic cell line was approximately 9-14 h (9). The elimination of CPEC occurs via deamination of CPEC to cyclopentenyl uracil (CPEU) (9, 62, 63), a process catalyzed by cytidine deaminase. Although deamination contributes to the elimination of CPEC (62), the majority is eliminated in humans and other mammals through renal clearance (63, 64).

Incubation experiments with CPEC have shown that CPEC-TP induces depletion of CTP and dCTP in several solid tumor cell lines (colon carcinoma, neuroblastoma, glioblastoma) and hematological malignancies (myeloid and lymphocytic leukemia) (7-12, 51, 60, 65-71). The decreased concentrations of CTP and dCTP were paralleled by diminished DNA and RNA synthesis (7, 9) and/or a growth-inhibitory effect (7-10, 12, 51, 60, 65, 66). It has been shown in a colon carcinoma cell line that CPEC-TP is incorporated into RNA but not into DNA (60). In primary cell cultures of samples of pediatric patients with ALL or AML, a major decrease in CTP and dCTP synthesis was observed after incubating these cells for 18 h with submicromolar concentrations of CPEC (48, 53). This was confirmed in cell cultures of samples obtained from adults with ALL, with a 50% growth-inhibitory concentration of 12 nM for CPEC (72).

In *in vivo* studies in mice, CPEC exhibited an antitumor effect (8, 66, 72, 73). CPEC-induced toxicity could be reversed by administering cytidine 4 h after the CPEC infusion, while the antitumor effect was not compromised by the addition of cytidine (66). The therapeutic window of CPEC could be increased by sequential administration of cytidine, which may be important since in some mouse strains the toxicity of CPEC may be a concern, as shown by Schimmel *et al.* (72). This observation of toxicity warrants additional experiments, since only two ALL *in vivo* models were used in these studies (72). Furthermore, CPEC was well tolerated in other nonhuman primates, as discussed below.

CPEC has been studied in mice, rats (64), beagle dogs (69) and monkeys (62). In beagle dogs, increasing doses (200-800 mg/m$^2$) of CPEC resulted in macroscopic oral lesions and a variety of microscopic changes in lung, heart, blood vessel, lymphoid organs, gastrointestinal tract, liver, pancreas, adrenal glands, testes, prostate and mammary glands (74). In monkeys, a bolus of 100 mg/m$^2$ of CPEC was well tolerated, while a continuous infusion of CPEC leading to a steady-state plasma concentration of 1.25 μM resulted in only moderate neutropenia (62).

2. Therapeutic potential of CPEC

CPEC was studied in a phase I trial in adults with solid tumors, predominantly colon carcinoma (63). Hematological toxicity was dose-limiting at steady-state plasma concentrations > 2.5 μM, but was mild or absent at plasma concentrations < 1.5 μM. Gastrointestinal toxicity was mild to moderate and reversible. The major toxicity in the trial consisted of severe cardiovascular effects that occurred at steady-state plasma concentrations > 1.5 μM (63). Two of the 26 patients died due to irreversible hypotension and 3 others experienced reversible (orthostatic) hypotension. Symptoms of cardiotoxicity occurred 24-48 h postinfusion and were characterized by low voltages on the electrocardiogram (without signs of myocardial infarction) and vigorous ventricular contractions. The symptoms did not respond to intravenous crystalline fluid administration or dopamine in 2 patients. *Post mortem* examination was performed in 1 patient, revealing subendocardial necrosis (63). The pathogenetic mechanism of the cardiovascular toxicity has not yet been elucidated, but analysis of the clinical data from the phase I trial indicated that the symptoms are probably caused by cardiac toxicity rather than dysregulation of vascular tone.

A possible explanation for the cardiac toxicity induced by CPEC has emerged from the observation that rat cardiomyoblasts treated with CPEC showed an alteration in the metabolism of cardiolipin, a major phospholipid in heart cells and an essential element of the inner membrane of mitochondria (75). This alteration in cardiolipin metabolism could be reversed by the addition of cytidine (75). CPEC does not seem to provoke decreased contraction in isolated rat heart models (76), nor does it induce apoptosis in cardiomyocytes (76). Therefore, the mechanism underlying the hypotension seen in humans remains to be explained and may not be related to cardiotoxicity but rather to vascular changes after exposure to CPEC. For other chemotherapeutic agents such as anthracyclines and taxanes, the mechanism of cardiovascular side effects is not fully understood and may be of multifactorial origin (77). Moreover, one should bear in mind that the hypotension observed in the phase I clinical trial was only seen at steady-state plasma concentrations exceeding 1.5 μM (63) and in adults, who generally have a different cardiac function compared to children. So far, 3 children and 1 adult with relapsed ALL treated with CPEC have had comparable or lower steady-state plasma concentrations compared to those reported from the phase I trial in adults with solid tumors (63), and only minor toxicity. In 1 of 4 patients with ALL, disease stabilization was observed. Therefore, the dosing schedule of CPEC in humans and in primates is of utmost importance in order to increase the therapeutic efficacy and decrease the side effects.

3. CPEC as a modulator of araC metabolism

Another application of CPEC may be to enhance the cytotoxic effects of cytarabine (cytosine arabinoside, araC) or gemcitabine. Cytarabine has been widely used for the treatment of acute leukemia for more than three decades (78, 79). It constitutes the main drug in almost all treatment protocols for AML in both adults and children, and has also proven beneficial in precursor B-cell ALL and T-cell ALL (80). Gemcitabine is an established drug for the treatment of pancreatic and lung cancer.

CPEC may favor the phosphorylation of cytarabine and gemcitabine and increase their cytotoxic effects on DNA by the following mechanism as described for cytarabine. Cytarabine (araC) is metabolized to araCTP by three consecutive phosphorylating steps catalyzed by deoxycytidine kinase (dCK), NMP- and NDP-kinase, respectively (Fig. 3). The first enzyme (dCK) appears to be rate-limiting in human leukemia cells (39). The phosphorylation of cytarabine to araCTP can be counteracted by the enzyme (deoxy)cytidine deaminase, which converts araC to araU, and deoxycytidylate (dCMP) deaminase, which converts araCMP to araUMP. AraCTP is eventually incorporated into DNA as a deoxynucleotide analogue, where it exerts its cytotoxic properties by inhibiting DNA replication by interfering with the process of DNA chain elongation (81). Furthermore, araCTP has been shown to inhibit DNA polymerase α (82) and β (83), although results from a previous study shed doubt on whether this contributes signficantly to the cytotoxicity of cytarabine (84). Finally, high concentrations of araCTP may inhibit DNA repair mechanisms (85).

Deoxycytidine kinase is considered the rate-limiting enzyme in the phosphorylation of cytarabine (86) and the enzyme is inhibited by dCTP (86, 87), and to a minor extent by araCTP (88). Moreover, dCTP competes with araCTP for incorporation into DNA (82). As mentioned previously, dCTP can be synthesized either via the salvage pathway of deoxycytidine or indirectly from CTP via ribonucleotide reductase. Inhibition of CTP synthetase by

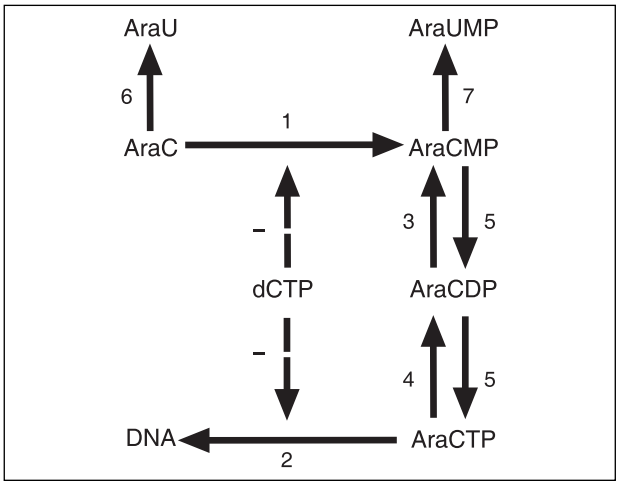


Fig. 3. Scheme representing intracellular cytarabine (araC) metabolism. The interrupted lines represent the inhibitory effect of deoxyCTP (dCTP) on deoxycytidine kinase (dCK) (1) and the competition of dCTP and araCTP for DNA polymerase (2). Nucleoside-monophosphate kinase (NMP-kinase) (3) and nucleoside-diphosphate kinase (NDP-kinase) (4) catalyze the second and third phosphorylation step of araC. Phosphatases (5), deoxycytidine deaminase (6) and dCMP deaminase (7) are catabolic enzymes involved in araC metabolism.

CPEC results in depletion of CTP and dCTP pools. Thus, by lowering the dCTP concentration, the formation of cytotoxic cytarabine metabolites may be enhanced, which has been demonstrated in a human colon carcinoma cell line, murine and human leukemia cell lines (10, 67, 69, 88, 89) and neuroblastoma cell lines (61).

## Conclusions

CTP synthetase is an attractive target for developing novel nucleoside analogues. The currently available inhibitor CPEC has cytotoxic effects *in vitro* and to a lesser extent *in vivo* which warrant further evaluation. The toxicity of CPEC in some species remains a concern, which may, however, be circumvented by using low to moderate doses. CPEC may have a cytostatic effect or may enhance the cytotoxic effects of other nucleoside analogues such as cytarabine or gemcitabine. These combinations could certainly play a therapeutic role in the management of ALL or AML, and even in solid tumors such as pancreatic cancer.

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