Expert Opinion

Development of cytarabine prodrugs and delivery systems for leukemia treatment

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Importance of the field: Cytarabine is a polar nucleoside drug used for the treatment of myeloid leukemia and non-Hodgkin's lymphoma. The drug has a short plasma half-life, low stability and limited bioavailability. Overdosing of patients with continuous infusions may lead to side effects. Thus, various prodrug strategies and delivery systems have been explored extensively to enhance the half-life, stability and delivery of cytarabine. Among the recent cytarabine prodrugs, amino acid conjugate ValCytarabine and fatty acid derivative CP-4055 (in Phase III trials) have been investigated for the treatment of leukemia and solid tumors, respectively. Alternatively, delivery systems of cytarabine have emerged for the treatment of different cancers. The liposomal-cytarabine formulation (DepoCyt®, Pacira Pharmaceuticals Inc., New Jersey, USA) has been approved for the treatment of lymphomatous meningitis.
Areas covered in this review: Various prodrug strategies evaluated for cytarabine are discussed. Then, the review summarizes the drug delivery systems that have been used for more effective cancer therapy.
What the reader will gain: This review provides in-depth discussion of the prodrug strategy and delivery systems of cytarabine derivatives for the treatment of cancer. The design of cytarabine prodrugs and delivery systems provides insights for designing the next generation of more effective anticancer agents with enhanced delivery and stability.
Take home message: Strategies on designing cytarabine prodrug and delivery formulations showed great promise in developing effective anticancer agents with better therapeutic profile. Similar studies with other anticancer nucleosides can be an alternative approach to gaining access to more effective anticancer agents.

Keywords: amino acid, arabinofuranosylcytosine, araC, chitosan, cytarabine, delivery system, fatty acid-cytarabine, liposome, nanoparticle, prodrug

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1. Introduction

Blood cancers such as leukemia, non-Hodgkin's lymphoma, Hodgkin's lymphoma and myeloma are characterized by the abnormal production of malformed lymphocytes and/or other blood cells. The cancer cells multiply without control and accumulate in bone marrow, blood and lymphatic tissues, and thus interfere with healthy cells and immune cell production and function. Ultimately these events result in blood/lymphatic disorders, such as leukemia, lymphoma, myeloma and myelodysplastic syndromes based on affected tissue [1].

The malformed cells are the result of one or more acquired mutations in DNA of lymphatic or blood forming stem cells, which on multiplication (clone formation) generate a high number of abnormal white blood cells. For example, in chronic myelogenous leukemia (CML) acquired mutation affects hematopoietic stem cells.

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Article highlights.

- The paper reviews cytarabine and therapeutic applications alone or in combination with other drugs, mechanism of action and metabolism, and two main strategies, prodrug and delivery systems, for improving the stability, half-life, duration of action, or bioavailability of the drug.
- Prodrug strategies included conjugation with fatty acids, amino acids and substituted-phosphate derivatives of cytarabine.
- In delivery systems, the most commonly used approaches include hydrogels, liposomes, niosomes, nanoparticles and polymers.
- Both prodrug and delivery systems have been effective at improving the biological profile of cytarabine. Several drugs or candidate compounds or formulations have been introduced, such as Valcytarabine, CP-4055 (Elacyt) and liposomal-cytarabine formulation (DepoCyt).
- Design of cytarabine prodrug derivatives and use of delivery systems have generated prodrugs or formulations that have optimal anticancer activity, stability, or delivery.

This box summarizes key points contained in the article.

CML is characterized by the presence of a Philadelphia (Ph) chromosome generated through translocation and fusion of the Abelson oncogene (ABL) at chromosome 9 and the breakpoint cluster region (BCR) at chromosome 22 leading to the generation of a BCR-ABL fusion oncogene, which in turn translates into a Bcr-Abl oncoprotein [2].

Leukemia, lymphoma and myeloma are diagnosed in all ages and accounted for the deaths of ~ 54,000 people in the US in 2010, nearly 9.5% of deaths from cancer in 2010 so far. Leukemia is one of the most fatal hematopoietic neoplasms and represents a wide range of blood cancers mainly related to an abnormal increase in the number of white blood cells (leukocytes). The death toll is expected to increase as new cases are diagnosed annually, suggesting a highly fatal nature of leukemia if unattended or untreated at early stages of development [3]. Thus, the causes, diagnosis, treatment and prognosis of leukemia are subjects of major interest [4].

Depending on the type of abnormal early blast or mature cells present, leukemia can be categorized into acute or chronic forms, respectively. Acute leukemia is identified by the rapid increase of immature white blood cells that crowd and accumulate around bone marrow, and thus induce the hemopoietic organ's inability to produce normal blood cells. Therefore, immediate and aggressive treatment is required in acute leukemia owing to rapid progression and accumulation of the malformed cells, which with time enter the bloodstream and invade other organs. Chronic leukemia is caused by the excessive build-up of abnormal mature white blood cells, which takes time to progress [5]. Further, leukemia can be lymphoblastic or lymphocytic leukemias (LL) and myeloid or myelogenous leukemias (ML) depending on the type of blood cell affected. The lymphoblastic or lymphocytic leukemias occur as a result of the cancerous modifications of bone marrow cells that are converted to lymphocytes. Lymphocytes have a role as the infection-fighting immune system cells. The myeloid or myelogenous leukemias result from the cancerous change of marrow cells that form red blood cells, platelets and white blood cells [6,7].

Owing to the heterogeneity of leukemia, treatment options for different classes of leukemias vary. The leukemia chemotherapy involves drugs from different classes ranging from kinase inhibitors to DNA synthesis inhibitors [8]. In many cases, a combination of two or more drugs is administered to patients to manage leukemia [9,10]. Radiotherapy is sometimes used along with chemotherapy in some types and stages of blood cancer, such as acute lymphocytic leukemia (ALL) and B-cell lymphomas [11,12].

Cytarabine (4-amino-1-β-D-arabinofuranosyl-2-(1H)-pyrimidinone, 1-β-D-arabinofuranosylcytosine, araC, Cytosar-U) is a pyrimidine nucleoside-based anticancer drug (**Figure 1**) with arabinose sugar widely used for the treatment of leukemia. Cytarabine is predominantly used against acute myelogenous leukemia (AML) and non-Hodgkin's lymphoma (NHL), chronic myelocytic leukemia (blast phase), ALL and erythroleukemia [13,14]. It may be used alone or in combination with other anticancer agents, such as daunorubicin, doxorubicin, thioguanine, or vincristine.

When cytarabine is used in combination with other anticancer drugs for the treatment of different leukemias and solid tumors [15,16], the drug combinations generally improve the cancer therapy by synergistic effect [17]. Cytarabine is used in induction therapy in combination with anthracyclines and in consolidation therapy at higher dose for AML patients. The combination of cytarabine with purine nucleoside analogues, such as fludarabine and cladribine, has been explored extensively in the treatment of patients with relapsed or refractory AML [18]. In Hodgkin's disease (HD) and NHL, cytarabine is used in conjunction with other drugs using different regimens, such as DHAP (cytarabine-cisplatin-dexamethasone) [19] and ESHAP (etoposide-methylprednisolone-cytarabine-cisplatin) [20] in patients with relapsed or refractory lymphoma. Cytarabine is used as the front-line drug for mantle cell lymphoma (MCL) [21]. Hyyper CVAD-MTX/Ara-c regimen (rituximab with hyperfractioned cyclophosphamide, doxorubicin, vincristine and dexamethasone plus methotrexate and cytarabine) is used for Burkitt's leukemia/lymphoma [22] and ALL [23].

Cytarabine acts on rapidly dividing cells and inhibits DNA synthesis at the S-phase of the cell cycle and also hinders progression of cells from the G1-phase to the S-phase. When inside the cell, cytarabine is converted into the triphosphate derivatives by kinase enzymes to show its cytotoxic effect. The exact mechanism of action of cytarabine triphosphate has not been elucidated but it appears to inhibit DNA polymerase by competing with deoxycytidine triphosphate [24] and thus reduces the cell replication.

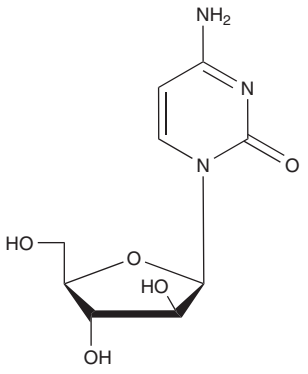


Figure 1. Chemical structure of cytarabine.

Cytarabine is a polar nucleoside and has a short plasma half-life. After three phosphorylation steps, cytarabine is converted to cytarabine triphosphate. It has been suggested that cytarabine triphosphate inhibits DNA polymerase by competing with the natural substrate, deoxycytidine triphosphate, resulting in DNA synthesis inhibition. The low bioavailability of cytarabine is created by its low permeability across the cell membrane and rapid conversion into inactive 1-β-D-arabinofuranosyluracil (AraU). The drug is rapidly converted into its uridine derivative by deamination of the cytosine ring in the presence of cytidine deaminase [25]. Thus, continuous intravenous infusion of higher doses is required to maintain a constant plasma level of the drug in 8 – 24 h. The higher doses of cytarabine lead to toxicity on normal organs and side effects [26]. Alternatively, cytarabine is administered by subcutaneous infusion in which peak plasma levels are attained after 20 – 60 min and decrease below steady-state level after 100 min [27].

Understanding the mechanism of the action and metabolism of cytarabine has allowed many investigations to improve the low bioavailability and stability. These efforts, depending on formulation and modification, can be divided into two major categories: prodrugs and drug delivery systems. The prodrug strategy for cytarabine involves the chemical modification or introduction of a potentiating group on the parent drug whereas the delivery system is involved in physical encapsulation of the drug without the introduction of any chemical modification to the parent drug. The molecules synthesized for prodrug evaluation may have higher therapeutic effect, whereas the delivery system may improve the effect in a particular tissue or organ. Various prodrug strategies explored and the potential of delivery systems for cytarabine are discussed.

2. Cytarabine prodrugs

Most of therapeutic drugs have to pass physiological barriers before reaching the target organ/tissue of action. Many drugs are modified into inactive derivatives during the transportation process. Among the various strategies used to minimize the undesirable metabolism of drug, the prodrug approach offers great promise in the retention of pharmacological properties of drugs, improving the drug efficacy, decreasing the toxicity, or reducing the dose administered.

Prodrugs are defined as pharmacologically inert chemical derivatives that can be converted into active drug molecules under *in vivo* physiological conditions through an enzymatic or non-enzymatic process, to exert a therapeutic effect at the desired target site. An ideal prodrug is converted to the original therapeutically active form of drug at the target organ and the substituted group is eliminated from the body [28]. Thus, the prodrug approach may minimize undesirable toxicity related to the parent drug in non-targeted organs [29,30].

Many prodrugs have been developed to improve drug efficacy, absorption, bioavailability, instability, drug delivery, drug specificity, toxicity, membrane transportation, or patient adherence (such as poor taste or odor of drug) [31,32]. The designing of prodrugs generally depends on many factors, including the mechanism of action, enzymatic interaction, lipophilicity, functional groups present on the drug and their lability, and metabolic pathway of the drug.

There have been major efforts in the development of cytarabine derivatives to generate compounds with higher therapeutic index for the treatment of leukemia and lymphoma (Sections 2.1 – 2.3). Among the strategies explored, the prodrug strategy of introducing modifications on the parent drug to enhance plasma half-life or delivery to cancer cells is a subject of great interest.

The prodrug design for cytarabine has been explored on the basis of mechanism of action and metabolism of the drug. The deamination of the cytosine base of cytarabine by cytidine deaminase leads to the formation of uridine derivative (AraU) and deactivation of the drug. In general, cytarabine prodrugs have been designed either to circumvent its catabolism into AraU or to enhance cellular delivery. Some of the prodrug approaches included designing amino acid-cytarabine, fatty acid-cytarabine and amino acid-fatty acid-cytarabine derivatives (Sections 2.1 and 2.2).

Mechanistically, cytarabine acts as a prodrug itself and is converted intracellularly to cytarabine triphosphate through monophosphate and diphosphate derivatives before generating biological activities. In the process, monophosphorylation is the rate-limiting step in the metabolic conversion to the triphosphate form. Alternatively, many phosphate derivatives of cytarabine have been evaluated as prodrugs to improve the cellular uptake of active metabolites (Section 2.3).

2.1 Amino acid derivatives

Many amino acid and peptide derivatives are known to increase cellular uptake of the drug molecules. Peptides with both nucleophilic (-NH_2) and electrophilic (-COOH) groups can be used for the conjugation of a large number of drug molecules. Amino acid or peptide attached to a drug can reduce the undesired metabolism by modification of the hydrolytic activity or the steric effect surrounding

susceptible functional groups. Cytarabine derivatives substituted with amino acids at 4-amino or 5′-hydroxyl positions have been synthesized and evaluated by different groups, as described below.

The amino acid-substituted derivatives of cytarabine at the 4-amino position were designed to reduce the metabolism of cytarabine into uridine derivative by blocking the proximity of cytidine deaminase. Derivatization of cytarabine with a single amino acid at an amino group reported by Jin *et al.* [33] involved the protection of hydroxyl groups by *tert*-butyldimethyl silyl (TBDMS) followed by conjugation with protected amino acids. Amino acid derivatives synthesized with arginine, leucine and isoleucine (**Figure 2**) were evaluated for cellular uptake in Caco-2 cells and were compared with that of cytarabine. The arginine derivative was shown to have comparable uptake whereas the leucine and isoleucine derivatives reduced the uptake of drug. They also compared the concentration dependency cellular uptake of the synthesized derivatives and found a similar uptake for cytarabine and arginine-cytarabine at increased concentrations, whereas leucine-cytarabine and isoleucine-cytarabine derivatives showed less cellular uptake than that of the parent drug at higher concentrations.

Sun *et al.* reported the 5′-amino acid derivatives of cytarabine and compared their cellular uptake in Caco-2 and HeLa cells (**Figure 3**) [34]. Among the various amino acid derivatives, the 5′-valyl prodrug showed highest permeability across the Caco-2 cells monolayer followed by the 5′-isoleucine derivative. Compared with the L-isomer of valine, the D-isomer had significantly less cellular uptake, suggesting that the stereochemical oligopeptide transporters may be involved in transporting amino acid prodrugs. Antiproliferative studies on the HL-60 cells showed the comparable activity of amino acid derivatives versus that of parent cytarabine. The antiproliferative effects of cytarabine and the amino acid ester prodrugs (IC_{50}) in HL-60 cells were reported as cytarabine (20 μM), 5′-L-valyl-cytarabine (16 μM), 5′-D-valyl-cytarabine (20 μM), 5′-L-isoleucyl-cytarabine (21 μM), 5′-L-phenylalanyl-cytarabine (17 μM), 5′-D-phenylalanyl-cytarabine (16 μM) and 5′-L-tryptophyl-cytarabine (19 μM). The study recommended the valyl prodrug as a potential candidate for the oral delivery of cytarabine in a similar fashion to the other valyl prodrugs that have been reported to be promising with other nucleoside drugs for other conditions [35].

2.2 Fatty acid derivatives

The pharmacological properties of drugs depend on overall surface interactions with medium and can be modulated by variation in the lipophilicity/hydrophilicity ratio. The presence of long fatty acyl chains makes the drug molecules lipophilic [36]. Furthermore, the lipophilic drugs pass across the membrane rapidly and make the drug available for biological action at higher intracellular concentration in target cells. The fatty acid conjugation of the drugs through esterification or amidation leads to the formation of an ester or amide bond, respectively, with the drug molecule. The approach has two advantages. First, the conjugation protects the functional group. Second, the substitution allows sustained and continuous release of the drug through hydrolysis, thus minimizing the side effects at higher doses of drugs.

The prodrug approach using fatty acid derivatization at the 5′-OH and 4-NH_2 groups of cytarabine has been evaluated by many research groups. In addition to making the drug more lipophilic, the strategy may also allow the protection of cytarabine from cytidine deaminase enzyme action, thus preventing its metabolism into the inactive form AraU.

Fatty acid presence along with amino acid further utilizes the assumed properties of amino acid as well as fatty chains. Liu *et al.* have used the advantage of simultaneous amino acid and fatty acid derivatization [37]. They reported N4-amino acid (Val, Met, Tyr, Glu and Arg) and fatty acid derivatives with chain lengths of 10, 14 and 18 carbons (**Figure 4**). The synthesis was accomplished through the direct coupling of amino acid-fatty acyl derivatives and cytarabine at the 4-NH_2 (i.e., N4) position through a peptide bond. The synthesized derivatives showed variation in antiproliferative activities with different amino acids and/or fatty acid. The studies performed using HL-60 and HeLa cells showed potential reduction in IC_{50} with the substitution at N4. Among the different amino acid derivatives, the methionine derivative demonstrated better antiproliferative activity when compared with the other synthesized conjugates. The antiproliferative activity was dependent on the chain length and decreased with increase in chain length at N4, suggesting that the introduction of bulky groups at N4 through the amide bond would not be beneficial in improving biological activity.

Fatty acid-substituted conjugates of cytarabine at 5′-OH showed significantly different behavior from N4 derivatives. Derivatization with different saturated and unsaturated higher fatty acids showed comparable activity to that of cytarabine in different cell lines [38]. Among the derivatives, CP-4055, a fatty acid derivative of cytarabine (ara-C-5′-elaidic acid ester) (**Figure 5**), facilitated and enhanced cellular accumulation and retention of ara-C in tumor cells. Unlike cytarabine, the cellular uptake of CP-4055 is independent of nucleoside transporters, and is believed to be by means of passive diffusion through the cellular membrane or an alternative internalization mechanism [39]. CP-4055 is then hydrolyzed intracellularly by esterases to release free cytarabine, which is subsequently phosphorylated to the active triphosphate analogue. When compared with cytarabine as a potent inhibitor of DNA synthesis [40], CP-4055 also transiently inhibits RNA synthesis. Furthermore, the fatty acyl conjugation potentially delayed inactivation to ara-U. CP-4055 is not a substrate for cytidine deaminase. CP-4055 demonstrated cytotoxicity in solid tumor and leukemia cells *in vitro* and *in vivo* [41]. CP-4055 was evaluated against a lymphoma cell line with deficient nucleoside transport (5CEM-araC/C8) and was resistant to cytarabine. CP-4055 was able to kill a high proportion of drug-resistant cells by apoptosis [42].

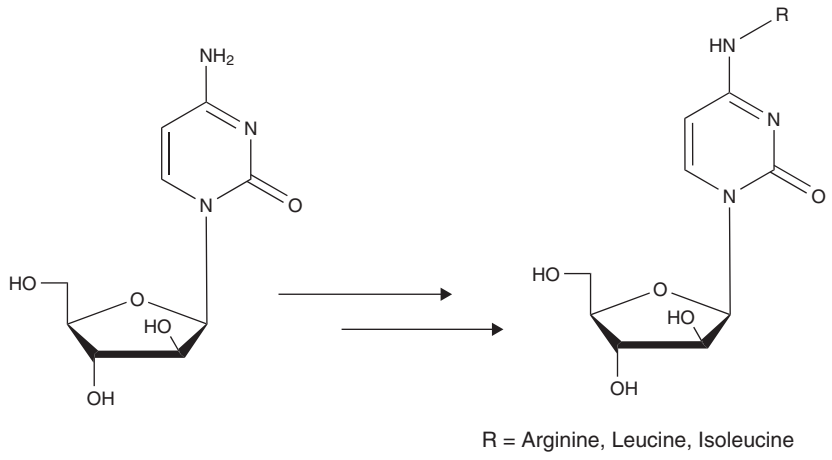


Figure 2. N4-substituted amino acid derivatives of cytarabine.

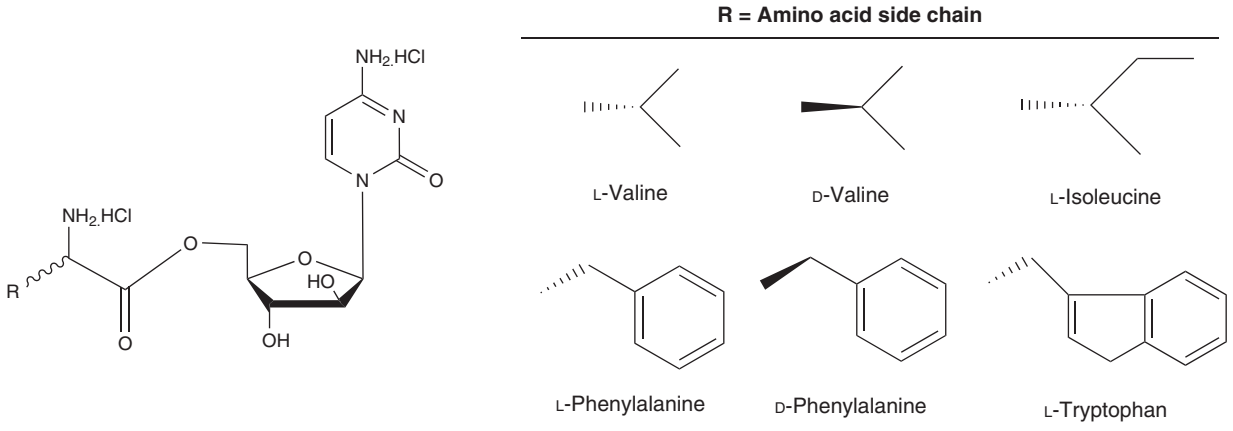


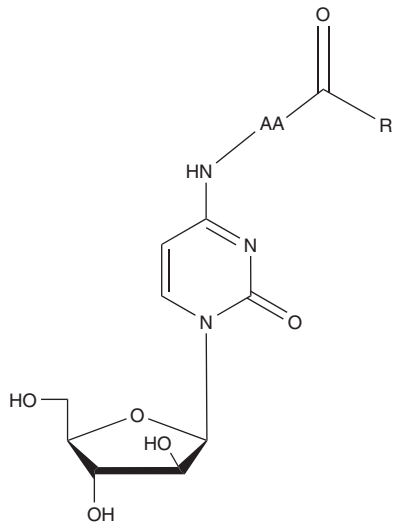
Figure 3. Structure of 5′-amino acid ester prodrugs.

Further studies of CP-4055 in human leukemia and lymphoma HL-60 and U937 cells demonstrated additive or synergistic cytotoxic activity in combination with other anticancer drugs, such as cloretazine, idarubicin, gemcitabine, irinotecan and topotecan [43].

Continued treatment with CP-4055 showed enhanced antitumor effect in human leukemia and in solid tumor models *in vivo*. The elaidic acid derivative of cytarabine was studied in Phase I studies in patients with solid tumors, such as malignant melanoma, ovarian cancer and non-small cell lung cancer. The pharmacokinetic study in 34 patients demonstrated that at dose levels of > 150 mg/m^2 after 0.5 – 2 h intravenous infusion, CP-4055 was well tolerated and remained in plasma for up to 5 – 10 h [44]. CP-4055 under the brand name Elacyt™ has now moved to Phase III trials (NCT01147939) by Clavis Pharma (Oslo, Norway) in patients with late stage AML.

The 2′-hydroxyl position of arabinose sugar in cytarabine was explored further for functionalizing with fatty acids. The authors synthesized and evaluated the 2′-*O* and 5′-*O*-myristoyl and 2′,5′-dimyristoyl derivatives (**Figure 6**). As the cytotoxic mechanism of cytarabine involves phosphorylation of 5′-hydroxyl into triphosphate, the effectiveness of fatty acyl ester derivatives depends on the concentration of the parent drug delivered intracellularly after hydrolysis and availability of the 5′-hydroxyl group for phosphorylation [45].

The 5′-substituted fatty acyl derivative of cytarabine was not able to inhibit significantly the proliferation of leukemia cells (CCRF-CEM), even after 96 h at a concentration of 1 μM. On the other hand, 2′,5′-dimyristoyl derivatives of cytarabine and 2′-fatty acyl derivatives of cytarabine inhibited the growth of cancer cells by ~ 36 – 76% at a concentration of 1 μM after incubation for 96 h. 2′,5′-Disubstituted derivative showed comparable activity to that of parent drug cytarabine and physical mixtures after 96 h. Enhanced cytotoxic activity of 2′,5′-disubstituted derivative after 96 h compared with that in 24 h indicates that the conjugate releases cytarabine slowly and may behave as a prodrug for sustained delivery of the



AA = Val, Met, Tyr, Glu, Arg
R = $-(CH_2)_8CH_3$, $-(CH_2)_{12}CH_3$, $-(CH_2)_{16}CH_3$

Figure 4. Structure of fatty acid-amino acid-cytarabine derivatives.

parent nucleoside. Although the anticancer activity of the compound was only comparable to that of cytarabine after 96 h, the sustained-release property may be beneficial.

2.3 Phosphate derivatives

The phosphate prodrug approach has been widely used with nucleoside drugs [46]. Nucleosides are converted intracellularly into the monophosphate, diphosphate and triphosphate derivatives, respectively, before generating biological activities. The monophosphorylation is generally the rate-determining step and thus delivery of monophosphorylated nucleosides that bypass this step is highly desired. Cytarabine is also converted into the triphosphate form before incorporation into nucleic acids. Thus, various phosphate derivatives of cytarabine have been synthesized and evaluated, as discussed below.

Lipophilic phosphate prodrugs of cytarabine were designed to contain both the phosphate and lipid (fatty chain) properties. The concept of using lipophilic nucleoside monophosphate ester derivatives of cytarabine was proposed to bypass the first phosphorylation step and circumvent tumor resistance associated with decreased deoxycytidine kinase activity via kinase bypass. Furthermore, metabolic degradation by cytidine deaminase was expected to be reduced. Finally, partial masking of the phosphate charge and increased lipophilicity may allow higher intracellular levels of the parent analogue [47-49].

Initially, it was expected that cleavage of the prodrug moiety was a result of the action of phosphodiesterase; however, it was subsequently demonstrated that the release of the parent drug occurs by means of successive two-carbon degradation via oxidation in peroxisomes [50]. The investigations led to discovery of the stearyl phosphate diester of cytarabine (cytarabine ocfosfate, YNK01) (**Figure 7**) [51]. Further clinical evaluation of cytarabine ocfosfate showed enhanced stability and higher half-life when compared with cytarabine and showed great promise for oral administration [52-54]. A Phase I/II study demonstrated that 15.8% of the total dose was absorbed and metabolized to cytarabine and AraU [55]. Cytarabine ocfosfate was approved in 1992 [56] in Japan by Nippon Kayaky under license from Yamasa Shoyu for use in patients with adult acute non-lymphocytic leukemia and myelodysplastic syndrome [57].

Among other phosphate derivatives, the 5′-protected phosphate triester derivatives of cytarabine have been also evaluated as prodrugs. Gouy *et al.* synthesized mixed phosphate triester derivatives of cytarabine (**Figure 8**) [58], which were expected to release intracellularly cytarabine monophosphate after esterase- and phosphodiesterase-mediated hydrolysis, respectively (**Figure 9**).

Whereas other phosphotriester derivatives with other nucleoside drugs, such as AZT containing *S*-acyl-2-thioethyl (SATE) phenyl pronucleotides, have shown improvement in activity versus their parent nucleosides, these protected phosphate triester derivatives showed reduced activity against the leukemia cell lines when compared with that of cytarabine. The reduced antitumor activity observed with phosphotriester derivatives of cytarabine was assumed to be a result of the limited cellular uptake, a reduced hydrolysis to the nucleoside monophosphate, extracellular hydrolysis to nucleoside monophosphate, or an unexpected decomposition mechanism that hampered the intracellular formation of the 5′-mononucleotide (araCMP).

To compare the hydrolysis of phosphotriester derivatives of nucleosides [58], three derivatives, cytarabine phosphotriester derivative, AZT phosphate prodrug and snake venom phosphodiesterase (SVP) substrate (*p*-nitrophenyl thymidinyl phosphate), were tested in the presence of SVP, a representative for the type I phosphodiesterase enzyme family at 37°C. The hydrolysis led to the formation of the corresponding nucleoside 5′-monophosphate (**Figure 9**) with half-lifes of 4.6 h for cytarabine derivative, 53 min for AZT derivative and 43 min for *p*-nitrophenyl thymidinyl phosphate. Thus, 2′-deoxynucleotides were hydrolyzed faster than the corresponding ribonucleotides, and the araC metabolite appeared to be a poor substrate for SVP when compared with AZT or thymidine. These data suggest that a slow hydrolysis to cytarabine monophosphate contributes to reduced antitumor activity.

Phosphoramidate derivatives of cytarabine were also shown to have reduced anticancer activity when compared with cytarabine. For example, a nitrofuran phosphoramidate derivative (**Figure 10**) reported by Tobias and Borch showed reduction in antiproliferative activity when compared with that of cytarabine [59].

Phosphoramidate derivatives of cytarabine metabolite 2-β-D-arabinouridine (AraU) (**Figure 11**) were evaluated by Mehellou *et al.* [60]. The rationale for this study was to

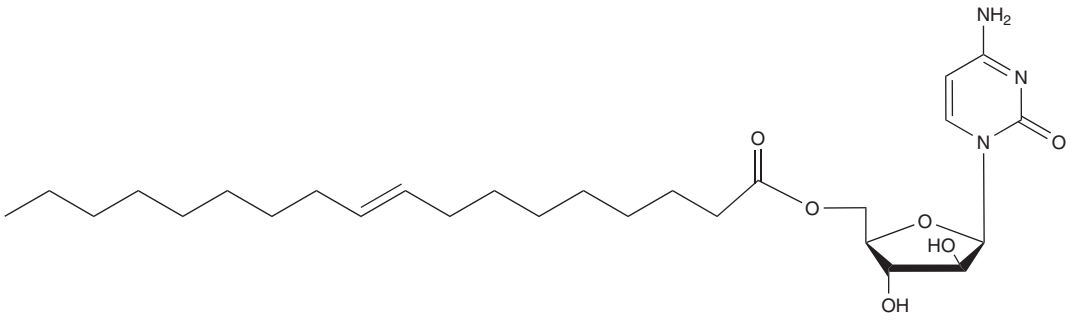
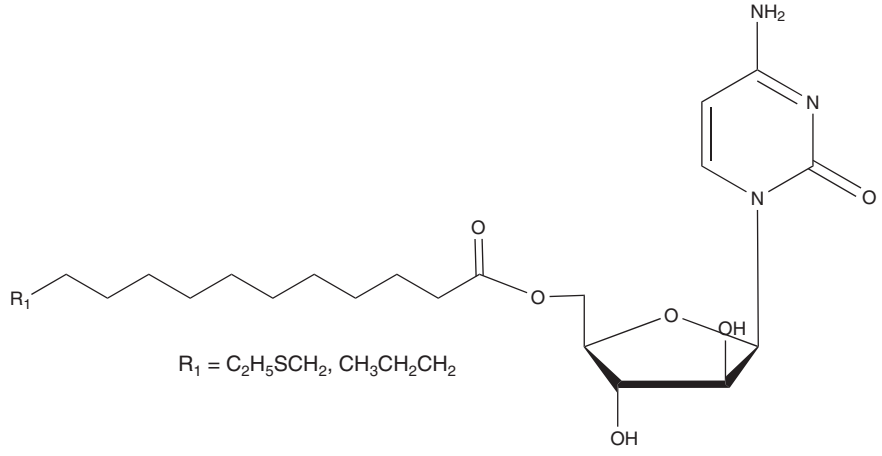
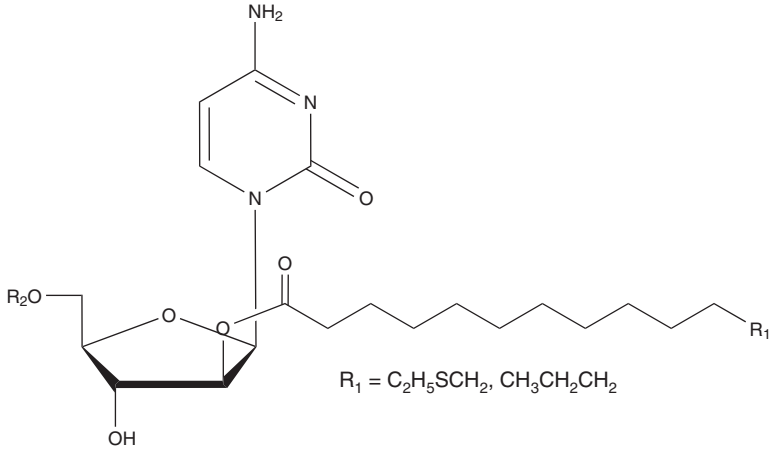


Figure 5. Structure of elaidic acid-cytarabine conjugate (CP-4055).



5′-Substitution



2′-Substitution R_2 = H
2′,5′-Disubstitution R_2 = myristoyl

Figure 6. Chemical structure of 5′-*O*-, 2′-*O*-monosubstituted and disubstituted cytarabine derivatives.

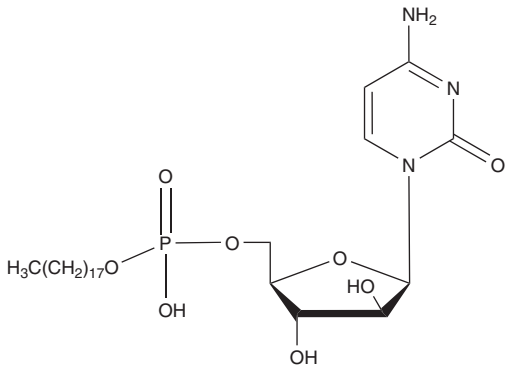


Figure 7. Chemical structure of cytarabine ocfosfate.

determine whether phosphorylation of AraU can activate the inactivate metabolite. Thus, masked phosphate prodrug was synthesized for intracellular delivery. The compounds were found to have reduced activity when compared with AraU. Hydrolysis studies in cell extract by NMR showed the reduced activity of phosphoramidate derivatives to hydrolysis because of the stability of the P–N bond to hydrolysis.

In general, the biological activities of synthesized fatty acid, amino acid and phosphate conjugates depend on the mechanism and ease of intracellularly released cytarabine or monophosphate derivative. The phosphorylation process occurs on the 5′-OH group of the arabinose sugar. Prodrug strategies that used either the substitution at the 4-amine group of cytosine base or the protected 5′-monophosphate derivatives have resulted in compromised activities against various cancers.

3. Delivery systems

The drugs, which have poor solubility, low cell permeability, fast metabolism and elimination, or require continuous dosages for a longer period of time, could be encapsulated in a protected sheath or fast delivery vehicle without any covalent or chemical modification in drug structure. Various vehicles, sheaths, particles or systems have been introduced as drug delivery systems for improving the delivery of drug molecules to the target organ in required dosages. Some of the drug delivery systems or vehicles include nanoparticles, polymers, hydrogels, organogels, niosomes and liposomes.

The nucleoside drugs are polar compounds and have generally low membrane permeability. Several delivery systems evaluated with anticancer nucleoside drugs have been reviewed recently [61,62].

Cytarabine is a highly polar water-soluble nucleoside. Various systems have been investigated for encapsulation and targeted delivery of cytarabine. The selection of cytarabine delivery systems [63] depends on their encapsulating capacity. Some include nanoparticles, hydrogels, chitosan, niosome vesicles of Tween-20 and liposomes. Liposomal cytarabine was approved by the FDA for the treatment of lymphomatous meningitis [64].

3.1 Hydrogels

Hydrogels are biomaterials consisting of a polymer matrix with a high water retention capacity. The hydrogels are capable of encapsulating the hydrophilic drugs. Sustained release of the drug depends on the polymer matrix constitution. The hydrogels have application as biomaterials because they have similarities in physical properties, such as water content, soft consistency and low interfacial tension, to those of natural tissues in biological systems [65]. The encapsulation of hydrophilic drugs such as nucleoside analogues with hydrogels would be easy and loading could be expected to be higher compared with other polymers. On the other hand, the strong interaction among the polymer and drug would hinder the release.

Cytarabine has been studied with HEMA (2-hydroxyethyl methacrylate) [66] and HEMA copolymerized with acrylamide [67]. The study showed that the rate and total time of cytarabine release from the copolymer (hydrogels) can be partially modulated by varying the amount of monomer composition and degree of crosslinking of the gels. Even though hydrogels have not been completely successful for the delivery of cytarabine, more studies with different polymer compositions can be used to find a balanced system for drug encapsulation as well as sustained release.

3.2 Liposomes

Liposomes or lipid capsids have been important vehicles for the delivery of several drugs [68]. The liposomes are lipophilic systems and can pass the cell membranes easily [69]. They possess unique pharmacokinetic characteristics because of their nanometer size (they range in mean diameter from 50 to 250 nm) as systemically administered vesicles [70]. These unique characteristic properties allow clearance through the reticuloendothelial system, which leads to a relatively long systemic circulation time, and hepatic and splenic distribution. Furthermore, the liposomes show preferential extravasation and accumulation at the site of solid tumors owing to increased endothelial permeability and reduced lymphatic drainage in these tissues [71]. The increased endothelial permeability has been defined as the enhanced permeability and retention effect [72].

Thus, liposomal delivery is a means to modify the pharmacokinetic and pharmacodynamic properties of anticancer agents [73], improving pharmacological properties, and reducing or modulating their toxicity profile [74]. Polar drugs once encapsulated inside the liposomes are transported across the membranes by a facilitated mechanism of liposome capsid. Liposomal cytarabine formulation has been studied with different liposomal compositions and hybrid systems with polymers [75].

The cytarabine encapsulated liposome has been studied with thermosensitive hydrogels [76]. The hydrogel was based on the biodegradable chitosan and β-glycerophosphate (C-GP), which are thermosensitive and act as a thermal trigger to regulate the drug release from encapsulation.

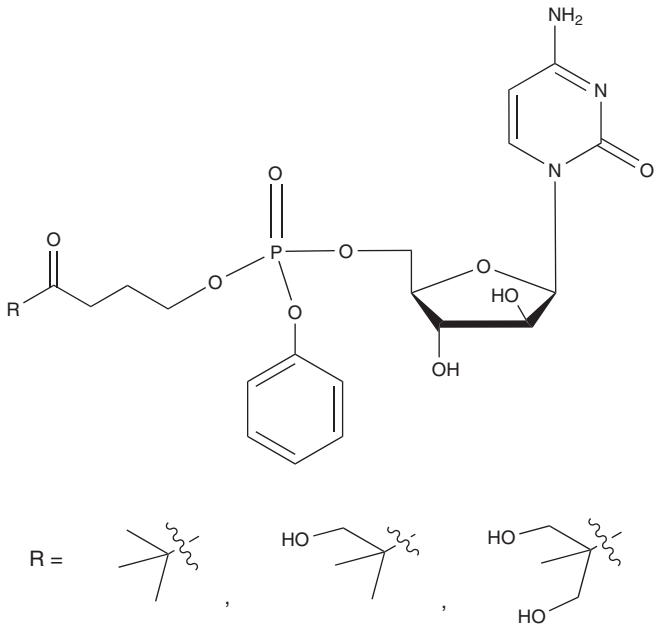


Figure 8. Chemical structures of mixed phosphate araC prodrugs.

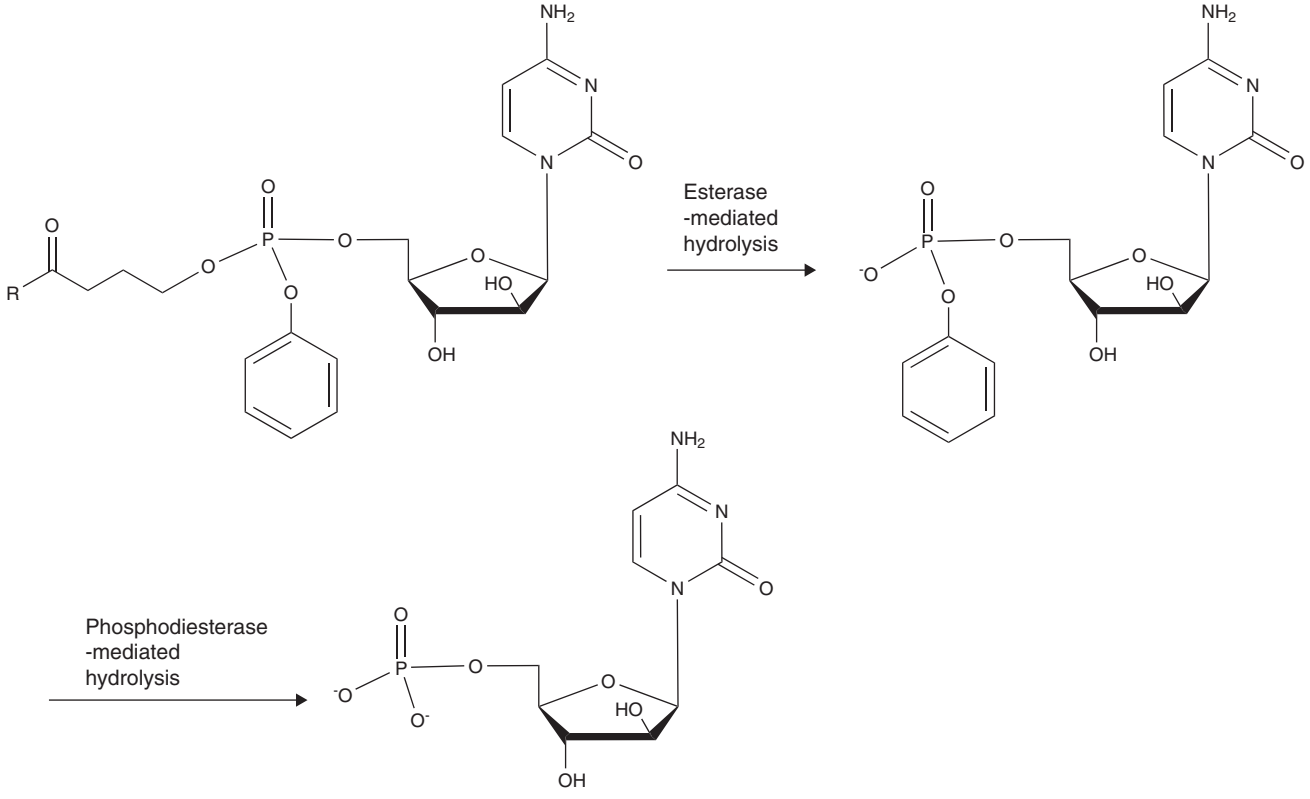


Figure 9. Possible mechanism of hydrolysis of a phosphotriester prodrug of cytarabine into a monophosphate derivative.

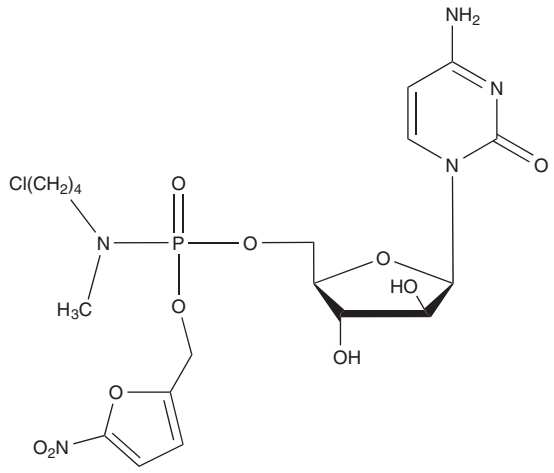


Figure 10. Chemical structure of a cytarabine nitrofuran phosphoramidate.

Combining liposomes with the C-GP hydrogel system gave the advantages of more sustained release of cytarabine along with a higher stability of the hydrogel-liposome system compared with liposome alone. The investigators suggest that the hydrogel system is appropriate for controlled and sustained release of cytarabine.

Cytarabine crosses the blood–brain barrier only to a limited extent after rapid intravenous administration. Liposomes with encapsulated cytarabine showed enhanced half-life and were found to be suitable for the treatment of lymphomatous meningitis. The formulation has been approved for the treatment of lymphomatous meningitis under the brand name DepoCyt® [77]. The DepoCyt formulation is prepared by encapsulation of a sterile suspension of cytarabine into multivesicular lipid-based polymeric liposomal particles composed of cholesterol, triolein, dioleoylphosphatidylcholine (DOPC) and dipalmitoylphosphatidylglycerol (DPPG). DepoCyt is administered as an intrathecal injection in cerebrospinal fluid for the treatment of neoplastic meningitis as a sustained-release formulation that maintains cytotoxic concentrations of cytarabine in cerebrospinal fluid for > 14 days following a single injection [78,79].

Cytarabine has been used in combination with anticancer drugs for synergistic antitumor activity. The combination of anticancer drugs as a cocktail is now commonly used clinically in cancer chemotherapy. Alternatively, two or more drugs can be encapsulated in the liposomes to enhance the delivery. The combination of cytarabine with daunorubicin HCl (a DNA intercalator) in 5:1 ratio in a liposomal formulation (cytarabine/daunorubicin HCl liposome injection) has been used by intravenous infusion for synergistic therapy in leukemia treatment as CPX-351 [80]. The liposome membrane is composed of distearoylphosphatidylcholine (DSPC), distearoylphosphatidylglycerol (DSPG) and cholesterol in a 7:2:1 molar ratio. The liposomal formulation (CPX-351) demonstrated enhanced therapeutic activity in preclinical tumor models [81] and promising anticancer activity in a Phase I clinical trial in patients with hematological malignancies [82,83].

The substituted derivatives of cytarabine have also been encapsulated in liposomes to improve the delivery and anticancer activity. Schwendener and Schott studied the encapsulation of N4-fatty acyl derivatives in liposomes and evaluated their potency compared with cytarabine liposomes [84]. The lipophilic N4-hexadecyl cytarabine (a potent derivative among N4-fatty acyl derivatives) was encapsulated in liposomes and evaluated in a cologenic assay with HL-60 cells, showing comparable activity to cytarabine. On the other hand, the liposomal formulation showed higher cytotoxicity in cytarabine-resistant cell lines compared with that of parent cytarabine [85]. The cellular uptake of liposomal cytarabine derivative was found to be five times more than cytarabine [86]. Among other derivatives of cytarabine, N4-octadecyl cytarabine also showed a similar behavior. After intravenous injection, the liposomal formulation showed longer plasma half-life compared with cytarabine [87,88].

3.3 Nanoparticles

Nanometer size particles have higher delivery through enhanced permeability to the tumor sites [89]. Drugs attached or encapsulated are delivered at higher concentrations to the site of action and are protected from fast excretion [90]. The polymeric nanoparticles have been investigated extensively for the delivery of various drugs [91,92].

Rukmani *et al.* investigated the methacrylic acid polymeric nanoparticle encapsulation of cytarabine [93]. Detailed morphological analysis of nanoparticles and further *in vivo* study of hematological parameters after administering CTH nanoparticles continuously for 9 days to tumor-bearing mice showed an improvement in lifespan of leukemia mice by the formulation when compared with cytarabine alone. The nanoparticular cytarabine formulation was able to restore the altered physiological parameters of leukemic mice to normal levels and was found to have improved the pharmacokinetics profile and prolonged the lifespan of mice, with increased white blood cell count, which make the nanoparticles a potential carrier for the cytarabine.

3.4 Other delivery systems

Among other systems that can serve as delivery vehicles, the natural or artificial polymers/molecules that are biocompatible have been evaluated to improve the delivery of the drug. Some systems include chitosan [94] and niosome vesicles [95] of Tween-20 and Tween-80 [96,97]. With these systems there has been little success in getting the final applicable system because of insufficient release of the drug from the conjugates.

In conclusion, among the delivery systems evaluated for application of cytarabine, the liposomal formulation has been successful at increasing the half-life and has found application in the treatment of lymphomatous meningitis.

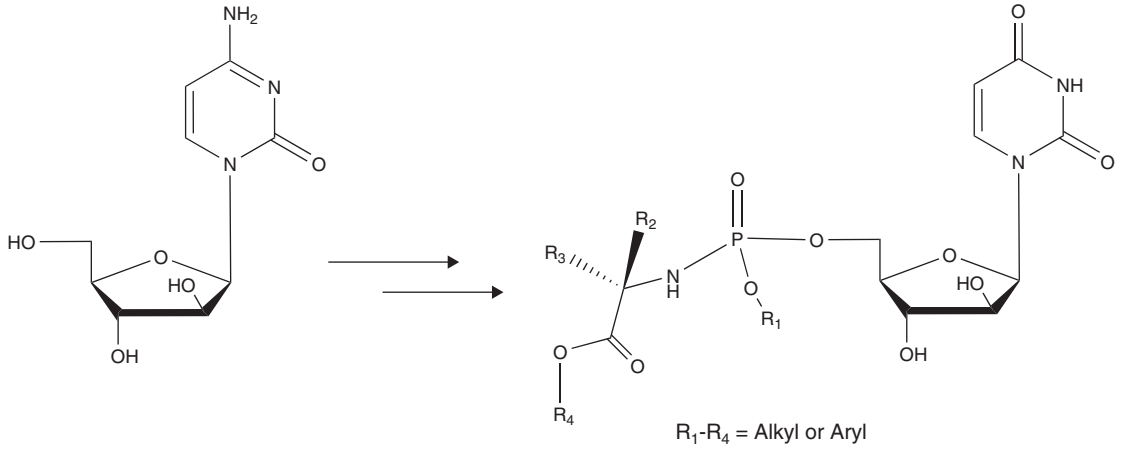


Figure 11. Chemical structure of AraUphosphoramidates.

The other systems have shown promising improvement in delivery and require more systemic study for final application.

4. Conclusion

Cytarabine, a polar nucleoside antileukemia drug with limited plasma stability and cell permeability, has been evaluated extensively by using prodrug and delivery systems. The prodrug attempts to improve delivery to target tissues, plasma stability and therapeutic effect have been successful in the generation of new drug conjugate molecules. Based on these concepts, several prodrugs and formulations were designed for cytarabine. Prodrug strategies included conjugation with fatty acids, amino acids and substituted-phosphate derivatives of cytarabine. The activities of these conjugates were dependent on the rate of hydrolysis, cellular uptake and resistance to cytidine deaminase. Among the prodrugs, amino acid derivative ValCytarabine and fatty acid derivative CP-4055 (under Phase II trials) are being evaluated for the treatment of leukemia and solid tumors, respectively. Among lipophilic phosphodiester derivatives, cytarabine ocfosfate has been approved for leukemia treatment. Furthermore, many delivery systems for improving cellular permeability, encapsulation and the sustained release of cytarabine have been evaluated. They include hydrogels, liposomes, niosomes, nanoparticles and polymers. The liposomal-cytarabine formulation (DepoCyt) has been approved for the treatment of lymphomatous meningitis. Both prodrug and delivery systems have been effective at improving biological profile of cytarabine. These approaches provide insights into designing prodrugs or delivery systems for other anticancer nucleoside drugs. Future investigations are required to generate more prodrugs and delivery systems with optimal biological activity and delivery. Furthermore, a combination of using an optimal cytarabine prodrug with an appropriate delivery system or using cytarabine or cytarabine prodrugs in conjunction with different anticancer regimens and/or delivery systems may be needed to explore further applications of these strategies in anticancer research.

5. Expert opinion

The concept of prodrug has been used successfully for improving the delivery, stability and pharmacological properties of drugs. The prodrug design is based on direct molecular changes on the drug to generate a dormant compound with appropriate physicochemical properties and desired biological activities. In the case of nucleoside analogues, the prodrug designing concept is also used to bypass the rate-limiting metabolic process by delivering nucleotides. It is not always easy to predict whether the dormant prodrug will be successful in generating the desired properties. As has been shown with many designs for cytarabine prodrugs, modulating higher stability and better release in target tissues still remain major challenges. Designing a diverse number of prodrugs has allowed the discovery of compounds with optimal stability and release properties.

Nucleoside analogues have sugar and base moieties. Conjugation at appropriate positions is required for designing optimal prodrugs. Cytarabine has an arabinose sugar with three hydroxyl groups and a base with a free amino group. These functional groups generate high polarity and a limited cellular uptake. Furthermore, some of the functional groups, such as 4-amino and 5′-hydroxyl groups, are critical in stability and activation of the nucleoside, respectively. Cytarabine is inactivated by deamination of the base. Thus, some of the methods used for designing prodrugs of other anticancer or antiviral nucleosides may not be used here because extra care must be taken to prevent deamination. On the other hand, cytarabine is activated by conversion into nucleotides through phosphorylation at the 5′-position. The designed prodrugs of cytarabine used the attachment of functional moieties at

either the amino group of the base or the 5′-hydroxy group arabinose sugar. The initial modifications used the fatty acids and different amino acids to improve the delivery and protection from deamination.

The rationale behind 4-N modification with introducing the amide group and bulky fatty acids and amino acids was to block enzyme-mediated deamination into uridine. Although the stability of the compounds was enhanced by reducing deamination, modifications at the N4-position led to reduction of activity of the compounds compared with the parent analogue. The more stable amide and the presence of a bulky group (amino acids alone and/or with fatty acid) at position 4 made the parent drug less active, possibly owing to the presence of steric hindrance at the base during incorporation in nucleic acids. Thus, improving the stability with N-4 substitution may not generate prodrugs with optimal biological properties.

Alternatively, the introduction of functional moieties at 5′-OH involved with hydrolyzable ester groups gave products with improved pharmacological properties. The 5′-fatty acid derivative (Elacyt) is in Phase III clinical trials for anticancer studies.

Furthermore, prodrugs with a substituted phosphate group were expected to enhance biological activity by bypassing the first intracellular rate-limiting phosphorylation step. A simple lipophilic phosphate diester prodrug, cytarabine ocfosfate, was approved in 1992 for leukemia treatment. However, the phosphoramidate derivatives with stable P–N bond showed reduced antiproliferative activity in most of the cancer cells, including leukemia and solid tumor lines. Thus, less stable phosphoester prodrugs are preferred to amidate counterparts for designing the next generation of lipophilic phosphate prodrugs of cytarabine.

Less research has been carried out on conjugation of cytarabine with hydroxyl groups at the 2′- and 3′-positions. It appears that substitution at the 5′-position with fatty acids alone is beneficial in generating higher lipophilicity and cellular uptake, but may slow down the metabolic phosphorylation of cytarabine. Alternatively, it may be possible to design prodrugs with masked phosphate at the 5′-position and lipophilic chains at the 2′- or 3′-positions. It remains to be seen whether this strategy can combine the benefits of both approaches.

Owing to limited cellular uptake of cytarabine, non-covalent methods using delivery systems or vehicles were expected to show great promise. These strategies will protect the chemical integrity of cytarabine and the compound is delivered in an intact form. The encapsulation into protected sheaths such as polymers, liposomes, hydrogels and niosomes was expected to provide the advantages of membrane transportation and continuous availability in the plasma over time. Among the evaluated delivery systems, the liposomal formulation was successful in enhancing the cytarabine half-life and treatment of lymphomatous meningitis. The hydrogels and niosomes were able to entrap polar cytarabine, but the release from these systems remained slow owing to strong interaction of drug with the sheath. Future studies need to focus on optimization of delivery sheaths, particularly hydrogel surfaces and nanoparticles, to generate controlled or sustained release and appropriate balance of encapsulation and release properties.

It remains to be determined whether an optimal cytarabine prodrug or those under evaluation (e.g., Elacyt) can be combined with promising delivery systems such as liposomes. Furthermore, cytarabine is also used in combination with other anticancer drugs in the treatment of patients. Further studies are required to determine whether a combination of cytarabine prodrugs with other anticancer agents or using anticancer cocktails containing cytarabine prodrug or cytarabine with an appropriate delivery system can have any beneficial therapeutic effects in the treatment of cancer patients.

In summary, the design of cytarabine prodrug derivatives and use of delivery systems have generated prodrugs or formulations that have optimal anticancer activity, stability, or delivery. The exploration of inactive prodrugs or not optimal delivery systems provided a deep understanding of the underlying factors responsible for inactivity or incompatibility in formulation that led to the development of the first generation of products approved by the FDA. Further improvements in design, modification in the drug molecule, or delivery sheath may lead to the second generation of compounds with broader applications. The same path may inspire investigators to design more optimized anticancer nucleosides and other anticancer agents using the knowledge gained here.

Declaration of interest

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