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Cytarabine conjugates with biologically active molecules and their potential anticancer activity

Minireview

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The presented review article deals with various conjugates of arabinosylcytosine (araC). This powerful drug that is routinely used in therapy of hematological malignancies has some shortcomings, which limit its use and therapeutic effects. These are low lipophilicity, low stability to degrading enzymes and need for biological activation through phosphorylation. Conjugating araC to another molecule is done with the intention of increasing araC stability and lipophilicity and possibly avoiding rate-limiting araC phosphorylation. An attachment of that another molecule, possessing its own biological activity, may result in formation of a conjugated molecule with new biological activities and better therapeutic potential. The review deals with various araC conjugates formed at the positions N^4, 2, 2', 3' and 5'. Biological activities and differences from araC of compounds formed by conjugation are also discussed.

Key words: Cytarabine, arabinosylcytosine, conjugates, biologic activity, anticancer activity

Antimetabolites of pyrimidine nucleosides are an essential part of therapeutic intervention in hematological malignancies [1, 2]. Additionally, some of them are used in patients with solid tumors. These substances, including Cytarabine (araC), serve as alternative substrates for enzymes metabolizing naturally-occurring nucleosides and nucleotides and they compete with natural substrates (natural nucleosides and/or nucleotides) for specific and non-specific metabolizing enzymes and other intracellular target sites [1, 2, 3]. The success in this competition results in cytotoxicity that can be used in cancer therapy for the benefit of the patient [4]. Even as antimetabolites belonged to the first drugs available for cancer therapy, their therapeutic usefulness as well as molecular structure makes them suitable for modification in the search of even more potent anti-cancer drugs today.

Among anticancer agents, araC possesses a prominent position, especially in treatment of hematological malignancies such as acute leukemias and lymphomas [1, 5]. The highest benefit for patients is proved to be achieved when araC is used in combination with other anticancer drugs. For example, in acute myeloblastic leukemia or in acute lymphocytic leukemia, araC is administered with doxorubicin (Adriamycin) [5]. Additionally, araC is a part of FLAI (fludarabine, araC, idarubicin) protocol in acute myeloid leukemias [6] and is combined with anthracycline in acute lymphoblastic leukemia [7] or with mitoxantrone in non-Hodgkin's lymphoma [8].

On the other hand, the use of araC in the treatment of solid tumors is limited. Additionally, the use of araC in human is accompanied by toxicity that includes myelosuppression (neutropenia, anemia and thrombocytopenia). Nausea, vomiting, diarrhea and mouth ulcers are related to GIT toxicity [4]. Consequently, the modifications of araC molecule are being actively investigated in the aim of more suitable therapeutic regimes and prevention of toxicity.

One option in the search for new anticancer drugs (araC-derived molecules included) that is being utilized by medicinal chemist and other biomedical scientist and clinicians in order to increase the usefulness of antimetabolite anticancer agents is to form their conjugates with other biologically active molecules. These new molecules then contain two substructures, both with important biologic and therapeutic activity. The conjugate molecule's breakdown usually liberates two active

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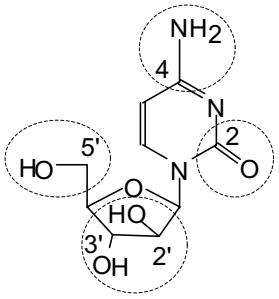

Figure 1. Structure of araC with marked positions suitable for conjugation

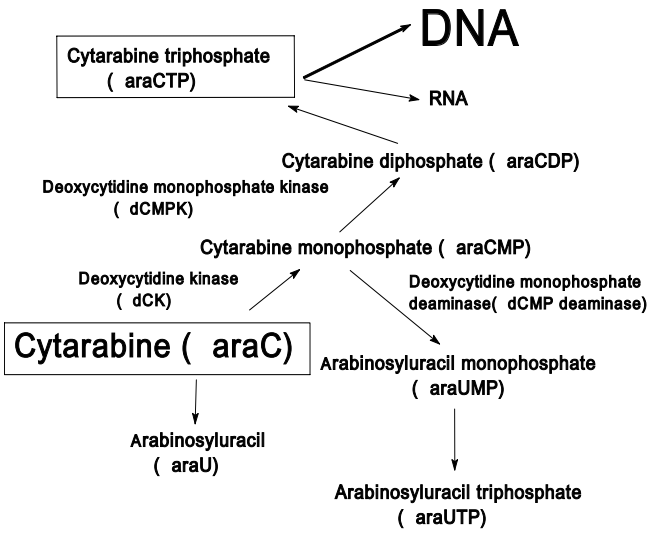

Figure 2. Cytarabine metabolism

molecular species. However, it cannot be excluded that in some cases, the conjugate may possess a distinct biologic activity even before the breakdown.

The goal of this review is to summarize scientific information on new conjugate molecules containing araC as a part of the conjugate molecule and evaluate the potential benefit of new conjugates' investigation and research from the point of view of their potential inclusion in future therapeutic regiments.

Properties of araC molecule – ability to form conjugates with other molecules. Cytarabine (cytosine arabinoside, 1-β-D-arabinofuranosylcytosine, araC) is an analogue of the naturally occurring pyrimidine nucleoside - cytidine [1–3]. It is formed by cytosine as a nucleobases and by arabinose that represents a sugar moiety. The change of the sugar moiety from ribose to arabinose may seem as not very significant from the chemical point of view as araC formed is just an isomer of cytidine. However, this relatively minor change of structure, consisting only of the change of the hydroxyl at the 2' position of the sugar moiety, has major consequences for biological utilization and properties of araC. This minor change stops the araC molecule from being a natural substrate for various cytidine-utilizing enzymes while making it an antimetabolite useful in anticancer (and antiviral) therapy (Fig. 1) [2, 3].

In principle, there are 3 sites at the araC molecule that are important for its metabolism, biological activity and that are important for derivatization and for, for the purpose of this review, conjugation with other biologically active molecules. The reactive sites of araC contain an amino group or hydroxyl (see Fig. 1). These groups make araC hydrophilic in nature and prevent the molecule from reaching part of a human body with higher lipid content. Consequently, this is the reason for araC being (successfully) used only in the treatment of hematological disorders.

The araC amino group is located at the carbon 4 of the pyrimidine heterocycle. It is important for anticancer activity as its deamination leads to the product, arabinosyluracil (araU, Fig. 2), which is without any anticancer activity. Consequently, forming conjugates at this position not only changes the pharmacokinetic properties of araC molecule but protects it also against metabolic deactivation [9–12]. This is very important as the excessive araC deamination leads to resistance in araC-treated patients.

The two hydroxyls on carbons at the position 2' and 3' are important for the overall functioning of the molecule. Normally, they are not affected during araC metabolism. However, their *trans* position is important for araC anticancer activity as it put this molecule apart from natural substrates cytidine and deoxycytine. Bulky attachments to these positions are very likely to interfere with araC utilization by kinases and other araC-utilizing enzymes.

The hydroxyl present at the position of 5' is essential for anticancer activity as this is a functional group that is phosphorylated by various cellular kinases (Fig. 2) to form an active molecule araCTP that as such is responsible for araC anticancer effect. The block of the 5' hydroxyl blocks anticancer effect of araC or highly diminishes it [13]. The formation of conjugates at this position may slow down the overall phosphorylation but may be beneficial in modifying araC pharmacokinetic [14], mainly by making the molecule more lipophilic.

It can be stated that inside human cells, normal and malignantly transformed, only N^4 amino group and 5' hydroxyl participate in metabolic processes. The amino group can easily be removed from araC and the 5' hydroxyl is phosphorylated. Basically, this is what happens with naturally occurring nucleosides cytidine and 2'-deoxycytidine. The sequence of actions performed by araC metabolizing enzymes is illustrated in Fig. 2.

The desired therapeutic outcome of araC metabolism is the formation of araCTP and consequent disruption of DNA synthesis through inhibition of DNA polymerases and incorporation into DNA chain resulting termination of chain elongation. These nuturally occuring therapeutic processes are decreased by the degradation process of deamination of

araC and araC monophosphate (araCMP) to arabinosyluracil (araU) and araU monophosphate (araUMP) [3, 4].

AraC conjugates. In some cases there may be no clear boarder between an araC conjugate and araC derivative. For the purpose of this review, an araC structure-containing molecule is regarded as a conjugate (and therefore included in this review) when it contains the araC structure without any substantial modification and when the added-to-araC part or conjugated part of the new molecule can exist in the biological systems as such. The added-to-araC part usually possesses substantial biological activity itself and is conjugated to araC in order to enhance araC anticancer activity, concentration or to modify its pharmacokinetics. Additionally, the majority of araC conjugates are able to liberate free araC from a conjugate molecule. It is important to mention that some new conjugate araC-containing molecules may act with an additional mechanism of action, which is not present in the two conjugate structures alone, but the main mechanism of action of araC and of the other component is normally not changed.

Conjugates of araC at the position of N^4 – the amino group. Conjugation of araC molecule through forming a bond with its amino group at N^4 position (see Fig. 1) is achieved relatively easily through preparation of araC amide with an organic acid. Forming a bond with an amino group is very important as it has two main consequences for involvement of araC into cellular metabolic processes. The first, it protects amino group from deamination [15, 16] preventing deactivation of araC and decreasing in its anticancer activity as it was shown that only minute amounts of product of deamination of araC, arabinosyluracil (araU), are phosphorylated. This provides an explanation for the lack of araU contribution to araC cytotoxicity [17]. The second, it changes pharmacokinetic, including cellular pharmacokinetic, parameters of the modified araC molecule [16] as new molecule is more lipophilic. Additionally, the molecules with modified amino group that originated from araC are as a rule not able to enter some metabolic process as a part of normal araC metabolism (phosphorylation at the position 5').

Many such derivatives cannot really be considered a conjugate as the organic acid used for the derivatization does not posses significant biological activity. Acetylated derivatives of araC [16] represent typical examples of such molecules. On the other hand, araC derivatives/conjugates became more interesting when the organic acid involved possess higher biological activity and when the resulting molecules differ substantially from parent araC or when the change of pharmacokinetics ensures that araC molecule reaches other, new cells or targets to exhibit its therapeutic activity.

Typical compound that reached some clinical prominence is behenoyl-araC (Fig. 3B). Behenoyl-araC is prepared by simple reaction of behenoyl chloride (chloride of behenoic acid, Fib X2, A) with araC. Behenoic acid as an organic acid contains 22 carbons in its molecule and no double bond. When behenoic acid forms a bond with a molecule of araC,

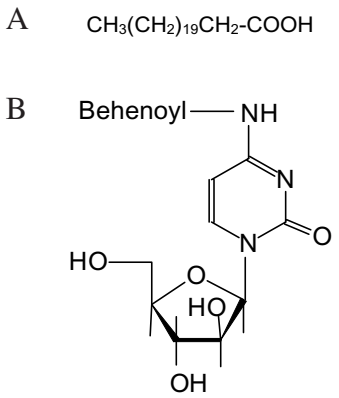

Figure 3. Chemical formula of behenoic (docosanoic) acid (A) and N^4-behenoyl-araC (B)

the resulting substance is highly lipophilic but liberates araC relatively easily in tissues where araC as such would not penetrate.

The information about behenoyl-araC was published in 1976 [18]. Its activity was tested in a murine leukemia L1210 together with other 50 compounds, all of them N^4-acyl derivatives of saturated fatty acids. Behenoyl-araC (NSC 239336), and also related N^4-stearoly-1-beta-D-arabinofuranosylcytosine, was superior to the parent araC, probably because of the resistance to cytidine deaminase and liberation of araC over a time period [19]. The efficacy of these compounds was also demonstrated in animal systems and it was shown that it is not affected by araC-inactivating cytidine deaminase [20]. It was also shown that acylation of araC does not change the mechanism of action of obtained substances as araC and a product of its deamination arabinosyluracil (araU) were found in preclinical study with monkeys in body liquids [21], and also, the presence of an active metabolite of araC, araC triphosphate (araCTP), was detected in blood of behenoyl-araC-treated patients [22]. It is important to mention that behenoyl-araC as such is not phosphorylated to the active araCTP but requires hydrolysis to behenoic acid and araC prior araC phosphorylation [23]. These findings make behenoyl-araC only a masked form of araC. On the other hand, it was demonstrated in some cancers, that even some araC-resistant ones, may be more sensitive to behenoyl-araC than to araC [24] probably due to changed pharmacokinetic parameters.

Despite the fact that behenoyl-araC or other araC fatty acid derivative [25] did not replace araC or got broad use, it is still considered in some therapeutic protocols. A study was published in 2006 analyzing the results of clinical use of salvage therapy including aclarubicin plus behenoyl-araC and prednisolone in previously-treated patients with acute myeloid leukemia AML [26]. The combination of aclarubicin and behenoyl-araC demonstrated a significant anti-leukemic efficacy in this group of patients and may worth of consideration when strategies for AML treatments are devised.

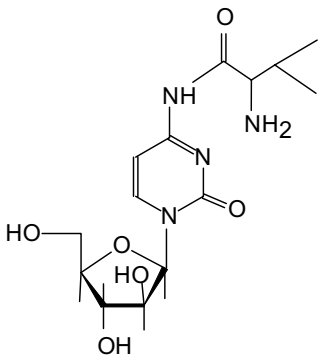

Figure 4. Chemical structure of L-valyl-araC.

Reactivity and properties of N^4 amino group of araC were also used for preparation of PEG-prodrugs of ara-C - poly(ethyleneglycol) based substances [27]. These prodrugs were based on preparation of disubstituted amides or a carbamate. The *in vitro* test showed superior activity of some PEG prodrugs to araC. Again, this is probably just because the liberation of free araC happened over some period of time.

Fatty acids were not only organic acids conjugated to araC. Preparation of peptiodmimetic prodrugs was reported [28] in order to increase cellular uptake of araC through involvement of various transporters. L-valyl-ara-C (Fig. 4) represents a substance investigated more in details. It was demonstrated that it interacts with multiple uptake transporters, including peptide transporters, organic anion and cation transporters and nucleoside transporters. Interestingly, it is possibly not transported by amino acid transporters [28]. As a result of interactions with various transporters, the accumulation of L-valyl-ara-C in Caco-2 cells was reported to be 5-fold higher compared to ara-C and it did not increase proportionally to the increased L-valyl-ara-C concentration. This, again, confirmed the active involvement of various cellular transporters in this drug cellular accumulation (the carrier-mediated transport).

More complex substances are represented by N^4-(acylpeptidyl)-araC (Fig. 5) [29]. Technically, they can also be considered an araC derivative. However, as a peptidyl moiety potentially possesses biological activity it, fulfils the 'requirements' for its classification as a conjugate. This type of compounds is not deaminated by cytidine deaminase. Additionally, these substances are bound via their hydrocarbon tails to membranes of phospholipidic nature. It was however determined that acylpeptidyl-araC containing only a tripeptide in its structure does not have an efficient enzyme/membrane contact due to shortness of a peptidyl moiety [29] but this can be solved by more detailed investigation into the effects of length and polarity of the peptide spacer attached to araC.

Another interesting araC conjugate is a substance obtained by conjugating araC with a peptide called peptide T [30]. Peptide T, a hydrophilic CD4-binding peptide, contains a following sequence of eight amino acids in its structure: Ala-Ser-Thr-Thr-Thr-Asn-Tyr-Thr [31]. The goal of synthesizing the conjugate of araC and peptide T was to selectively target CD4+ cells and increase araC concentrations within these cells. It was shown that peptide T-araC conjugates are capable of activating chemotaxis through binding to CD4 receptors on monocyte membranes. Peptide T-araC conjugates effectively inhibited growth of various leukemia lines at concentrations 4- to 10-fold higher than those of araC alone. However, their effect was not increased in CD4+ cell lines [31]. In general, peptidyl-araC substances did not succeeded as anticancer agents because of their low or no selectivity and higher inhibitory concentrations required in cancer cell systems.

Basically, the preparation of araC conjugates with poly-L-glutamic acid and polyN^5-(2-hydroxyethyl)-L-glutamine met

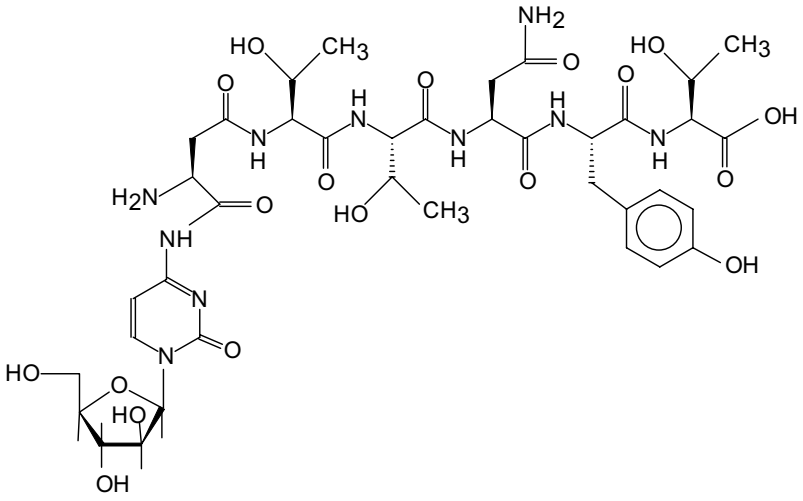

Figure 5. Chemical structure of a N^4-peptidyl-araC substance, particularly of H-Asp(Thr-Thr-Asp-Tyr-Thr-OH)-araC

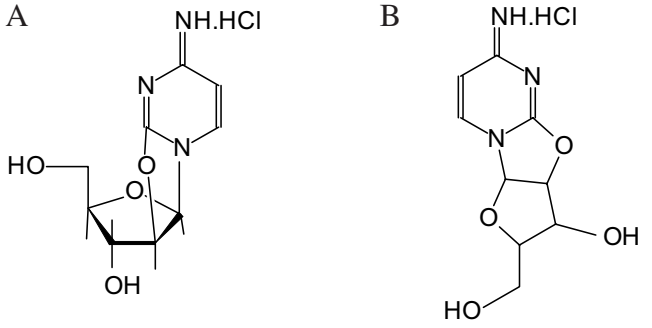

Figure 6. Chemical structures of cyclocytidine hydrochloride (2,2'-anhydro-araC) (a nucleoside-like chemical structure A and the same structure in the form B).

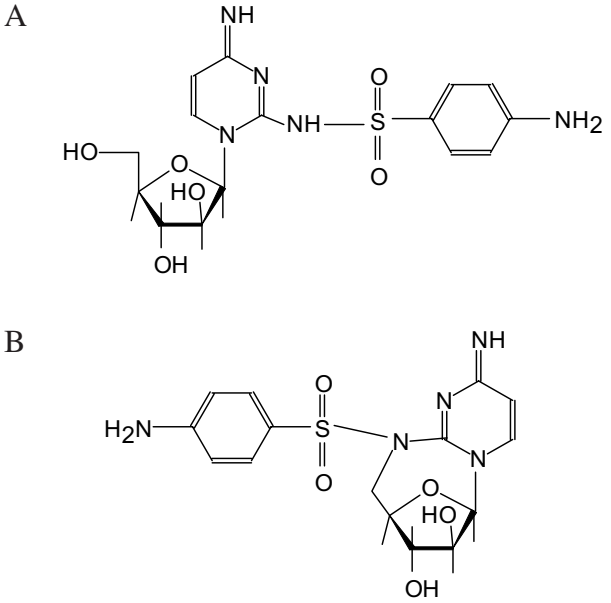

Figure 7. Chemical structure of araC conjugates with sulfanilamide.

with the same fate [32]. Some of the prepared conjugates increased life span of experimental animals in *in vivo* experiments by 170% when compared to equivalent araC dosage. However, the presence of any therapeutic advantage compared to the use of araC was not demonstrated.

Relatively high activity of araC conjugates was achieved through conjugating araC with polysaccharide molecules with carboxyl groups in their structure, for examples with polygalacturonic acid (PGA) and carboxymethylated yeast beta-D-glucan (CMG) [33]. In these cases, peptide bonds were formed through a coupling reaction that involved amino group of araC. It was demonstrated that these conjugates were hydrolyzed by trypsin and by trypsin-like proteases. This finding is important as these enzymes could participate in the hydrolysis of the prepared conjugates *in vivo*. The obtained *in vitro* anti-leukemic activity of the araC-polysaccharide conjugates reported was comparable or higher than the activity of non-conjugated/free araC combined with a polysaccharide [33]. The *in vivo* tests were performed with these substances using murine experimental model with animals bearing P388 and L1210 leukemia sensitive to araC and L1210 araC-resistant leukemia cells (L1210/araC) [34]. Single dose administration of PGA-araC or CMG-araC increased the survival time of experimental mice 1.5-times and 1.7-times compared with araC in L1210-leukemia-bearing animals. The fact that PGA-araC and CMG-araC were not active against araC-resistant leukemia line L1210/araC indicates that the mechanism of action of these conjugates (araC liberated from these conjugates) did not change. The observed effect was probably due to the prolonged release of free araC from conjugates through gradual hydrolysis.

In conclusion of this part of our review, it can be stated that the conjugates formed through bond with amino group of araC molecules as a rule do not demonstrate a change in mechanism of action of araC regardless structural characteristics of the other conjugated molecule or macromolecule. These modifications resulted in prolonged liberation of an active araC only. Consequently, therapeutic effects are enhanced in cases when the concentrations of araC and its active metabolite araCTP are sufficiently high for longer time period when compared to administration of free araC only. On the other hand, if the gradual liberation of araC into an organism results in insufficient concentration of araC, therapeutic effects decreased.

Conjugates at the position 2 – the carbonyl. The carbonyl group at the position 2 of the pyrimidine base (see Fig. 1) is important for the overall molecular properties of araC. It is involved in preparation of some cyclic nucleosides. The most significant one is cyclocytidine (2,2'-anhydro-araC, Fig. 6) [35, 36]. The 2-2' bond of cyclocytidine requires hydrolysis resulting in formation of araC [36] that is than responsible for the majority of therapeutic effects. However, it was shown that cyclocytidine as such possesses also biological activities of its own [37]. As cyclocytidine and other related molecules do not belong among conjugates, and also because none of these substances reached clinical prominence, the discussion of these molecules is beyond the scope of this review article.

Only few conjugates were reported in scientific literature that involved carbonyl and possessed anticancer activity. These were conjugates with sulfanilamide [38, Fig 7A and B]. As obvious from the Fig. 7B, both carbonyl and 5' hydroxyl are engaged in conjugation in the conjugate B.

The conjugation of araC and sulfanilamide is justified despite of the fact that anticancer activity of sulfanilamide itself was not reported. Sulfonamides, as a chemical group, are known to act as cell-proliferation inhibitors at mitosis [39]. They also arrest cell cycle in a specific phase, inhibit cancer-associated carbonic anhydrase and alter gene expression [40]. Sulfonamides celecoxib [41, 42] is already recognized for its usefulness in cancer therapy. These conjugates were investi-

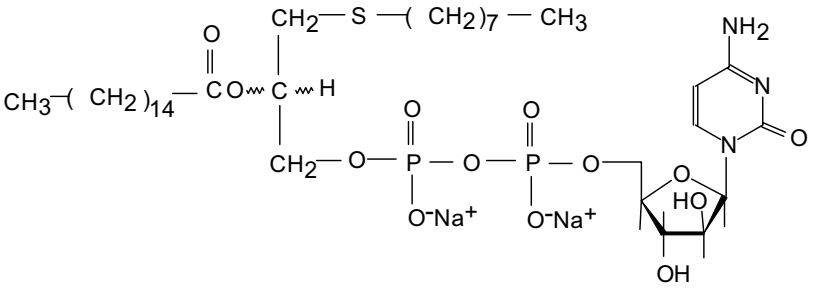

Figure 8. Chemical structure of a typical thioether phospholipid araC 5'-diphosphate-*rac*-1-*S*-octadecyl-2-*O*-palmitoyl-1-thioglycerol (araCDP-DL-PTBA).

gated because of expectations that protection of araC moiety against deamination and modifying its pharmacokinetic would be achieved while sulfonamide moiety may contribute by specific mechanisms to anticancer activity, for example by mediating cell-cycle arrest, activating caspases and downregulating COX-2 expression [42] or by interfering with enzymes, such as caspase anhydrase [43, 44] or with microtubule assembly dynamics [39, 44].

When tested *in vivo* [45], the conjugates were less active compared to araC but their enzymatic degradation was also decreased. The difference in cytotoxic and therapeutic activity between araC and two araC sulfanilamide-analogues was probably caused by their higher intracellular stability and slower liberation of araC from both conjugates. This protected araC from deactivation before it is liberated from a conjugate and extended the time period of therapeutic action of both araC conjugates. The testing of these substances in conditions with prolonged administration of these conjugates is being considered as both sulfanilamide-araC conjugates were not toxic. Their administration to experimental mice did not affect any important organ or function in the animals used at the dosage of 400 mg/kg of body weight [45]. This is an additional support for the hypothesis that tested araC conjugates retained the same mechanism of antileukemic activity as did araC.

The opening of 2,2' or 2,3' cyclic nucleosides by nucleophilic attack of sulfonamide anions was used in preparation of some additional C-2 sulfonamido pyrimidine nucleosides by Krizmanic et al. [46]. The prepared substances were tested in several human tumor cell lines *in vitro* with only moderate inhibition of tumor cells growth.

Conjugates at the position 2' or 3' – the hydroxyls. These two positions (see Fig. 1) are significant for determining whether a natural nucleoside or nucleotide can be used as a building block in a synthesis of DNA or RNA. In antimetabolites such as araC, presence of absence of various functional groups at the carbons at the positions 2' and/or 3' determines the interference with either DNA or RNA or both. However, no conjugates conjugating araC at these two positions are reported in scientific literature.

Conjugates at the position 5' – the hydroxyl. Conjugation at the 5' position (see Fig. 1) resulted in some very interesting compounds. The 5' carbon and the attached hydroxyl are essential for biological activity of araC because the activation of the araC prodrug molecule to the araC triphosphate happens at this position. On the other hand, blocking the 5' hydroxyl by conjugation prevents phosphorylation until free araC is liberated. This results in changed pharmacokinetic parameters, i.e. parameters of distribution, elimination and araC metabolism. The special attention was paid to conjugates of araC formed at the 5' carbon with lipids and phospholipids, other nucleosides, and some small molecules, for example steroids.

The attachment of a lipidic structure to araC is usually done through remains of phosphates as in araCDP (arabinosylcytosine diphosphate) that is a normal araC metabolite preceding the araC activation to its active metabolite araCTP (Fig. 2) or through some other small connecting molecules such as glycerol. The one of the earliest reports dealt with synthesis of 1-O-octadecyl-2-O-palmitoylglycerol and its 1-S-alkyl analogue [47]. Administration (i.p.) of a single dose (300 mg/kg) of conjugates of the ether and thioether lipids significantly increased life span of leukemia L1210-bearing mice by approx. 200%. These results were important as it was shown that chirality of the glycerol moieties is not critical for the activity. Another work dealing with thioether lipid conjugates of araC (1-S-alkylthioglycerols linked by a pyrophosphate diester bond to araC) [48] shown that this type of conjugates requires deoxycytidine kinase for activation of araC part of conjugate to an active araCTP metabolite. The fact that cytidine conjugates were ineffective against leukemia L1210 indicates that this type of conjugation does not change the mechanism of action of a conjugate compared to araC. This is also confirmed by the fact that these conjugates are not active against araC-resistant cell lines [48]. One of the more interesting substances of this type, 1-β-D-arabinofuranosylcytosine 5'-diphosphate-rac-1-S-octadecyl-2-O-palmitoyl-1-thioglycerol (ara-CDP-DL-PTBA) (Fig. 8) was shown to have effective antitumor activity in various transplantable tumors (colon 26 carcinoma, M5076 sarcoma) in mice [49].

A revival of this type of conjugates was brought by the papers published by Alexander et al. [50, 51] with phospholipid-araC conjugate (and also with phospholipid-gemcitabine

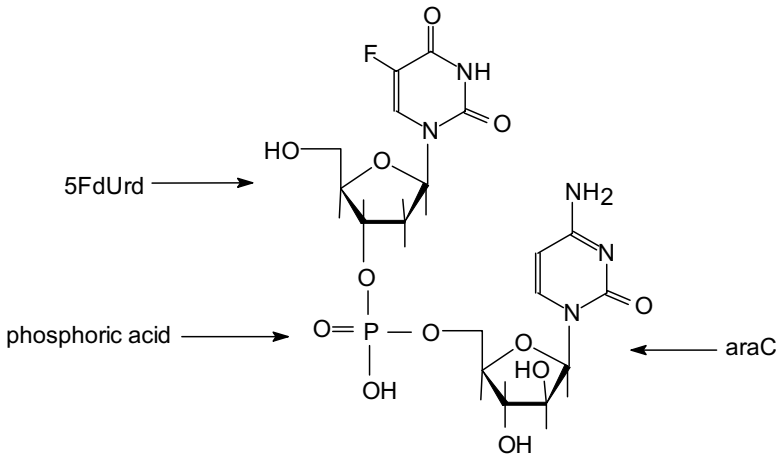

Figure 9. Chemical structure of a heterodimer or conjugate of araC and 5FdUrd connected by a phosphoric acid bridge.

conjugate). They demonstrated that araC is liberated from this type of conjugates by cleavage action of phospholipase C-like enzyme and the conjugates penetrate cellular membranes by passive diffusion due to higher lipophilicity of conjugate molecules compared to araC [50] and not via the human equilibrative nucleoside transporter (hENT1) [51]. It was also shown with gemcitabine conjugate that despite increased lipophilicity, this conjugate is not a substrate for the multidrug resistance efflux pump MDR-1, nor it requires deoxycytidine kinase (dCK) for the activation [51]. By this it was proved that conjugates may possess an ability to overcome some resistance mechanisms that render non-conjugate nucleoside antimetabolites ineffective in cancer therapy.

Recently, investigations of heterodimers of araC and 5-fluorodeoxyuridine (5FdUrd) or araC dimers [52] were reported. The two nucleoside molecules are usually connected by a bridge that contains remains of phosphoric acid and/or of glycerol. A conjugate usually contains araC conjugated at the positions 5' of its sugar moiety and the second nucleoside, for example 5FdUrd, is usually conjugated at its 3' position (Fig.9).

The rationale behind the synthesis of these conjugates or nucleoside dimers is to put together two nucleosides with different mode of action as this should increase therapeutic potential and damage to cancer cells. This is as these heterodimers should be more stable than individual nucleoside thus allowing for an increased accumulation of conjugated agent at solid tumor sites. Additionally, when phosphoric acid participate in formation of the bridge or bond between two nucleoside molecules that are being conjugated, those nucleosides connected to phosphoric acid at the position 5', when liberated from a conjugate, may not require phosphorylation to a monophosphate. This aspect of rationale for synthesizing such dimers or heterodimers is significant as this should overcome one of more usual chemoresistance mechanisms [53].

As reported earlier, exposure of multidrug-resistant cell lines [53] to araC dimers does not lead to an induction of P-170 glycoprotein expression. This suggests that such compounds circumvent MDR1 multidrug resistance. [53]. Therapeutic potential of such derivatives was also demonstrated *in vivo*. Moreover, it was also demonstrated that hetero conjugates of nucleosides connected by containing phosphate may be useful in assessing the type of acquired resistances [52]. Also, an araC-5FdUrd dimer was shown to override acquired chemoresistances when combination of araC and 5FdUrd exhibited only marginal activity [52]. This was shown once again to be due to the conjugate's ability to retain its anticancer potential, very probably by circumventing the necessity of the phosphorylation of nucleosides to a monophosphate that is a rate-limiting step of araC activation and is normally the major causal mechanism of araC resistance [54]. A very interesting study was published recently providing more insight into the affected downstream pathways participating in acquired chemoresistance of cancer cells to therapy [54]. It was also shown that a contribution to cytotoxicity and anticancer activity exhibited by a conjugate by conjugate-forming nucleosides differs [55].

Steroids-araC conjugates were the only small molecules conjugates deserving more attention. Specifically, the attention was paid to conjugates of araC and prednisolone and prednisone. A formation of a phosphodiester bond at the 5' position of araC was utilized successfully. The degradation of these conjugates led to the formation of araC monophosphate through an action of various enzymes but not of alkaline phosphatases [56]. The activity of prepared compounds in L1210 leukemia model was significantly higher than the activity of araC administered alone or in combination with a steroid. At the therapeutic regime employed [56], araC administration resulted in the increase of animal life span by 45%, administration of the same dose of araC in combination with equimolar doses of prednisolone or prednisone yielded

increase of the life span of experimental mice by 40 or 44%, respectively. On the other hand, the administration of prednisolone-araC or prednisone-araC conjugates resulted in the increase of life span of experimental animals by 89 and 100%, respectively [56]. With the understanding of araC mechanism of action we have today, the finding that these substances had only a marginal effect in araC-resistant line had to be expected [57].

Similar results were later obtained with other 5'-(steroid-21-phosphoryl)-1-β-D-araC in the same model [58]. The steroids used were 11-deoxycorticosterone, corticosterone, cortexolone, fludrocortisone, 6α-methylprednisolone and dexamethasone.

Conclusions

AraC is a very powerful molecule that is used for benefit of many patients as an anticancer agent in treatment of leukemias and lymphomas. However, it also has some significant disadvantages, such as low lipophilicity and low stability towards metabolic deactivation. Those limit its use just to treatment of hematological disorders. However, because of its excellent therapeutic activity, different variations of araC molecule are synthesized in order to improve araC physico-chemical properties. Conjugation, which is connecting of araC with other molecule – promoiety, is a way to modify araC lipophilicity and to protect it from actions of deactivating enzymes present in cancer cells.

So far, many conjugates of araC with other biologically active molecules were prepared as discussed above. However, none of them surpassed araC. Some of them got limited use, others, despite being relative active, did not enter clinics because of the fear of biological activities of their promoiety as in the case of steroidal araC conjugates.

Even if none of prepared and tested conjugates of araC got wider use, their synthesis elucidated properties of nucleoside and nucleotide molecules and elucidated some aspects of araC anti-cancer mechanisms of anti-leukemia activity. All of this is without a doubt generally highly beneficial for the development of chemical and bio-medical sciences and as such should continue.

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