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Amino Acid Phosphoramidate Nucleosides: Potential ADEPT/GDEPT Substrates

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Abstract—A series of aromatic, serum-stable, water soluble and nontoxic amino acid phosphoramidate monoesters of 5-fluoro-2′-deoxyuridine (FUdR) and 1-β-arabinofuranosylcytosine (Ara-C) was shown to inhibit the cellular growth of the human leukemia cell line CCRF-CEM in the presence of human prostatic acid phosphatase (hPAP).

The chemotherapeutic nucleosides 5-fluoro-2′-deoxyuridine (FUdR) and 1-β-arabinofuranosylcytosine (Ara-C) both function as antimetabolites. They possess potent antitumor activity and have been used since the 1960s for the treatment of acute myelogenous leukemia and various types of carcinomas. Unfortunately, like most anticancer agents, associated toxic side effects make their use dose limiting. Consequently, the ability to site specifically deliver nucleosides could dramatically enhance their therapeutic index by reducing the potential for subsequent systemic toxicities.

Over the last decade, several attempts have been made to generate an active drug from an inactive precursor, through the action of an enzyme present predominantly at the tumor site, with the goal of developing a new, less cytotoxic strategy for the treatment of cancer. Antibody and gene-directed enzyme prodrug therapies are two targeting strategies designed to improve the selectivity of antitumor agents. The approaches are based on the activation of a prodrug by an antibody–enzyme conjugate targeted to a tumor-associated antigen (ADEPT) or by an enzyme expressed specifically by tumor cells (GDEPT).[1,2] The ADEPT approach involves two steps. The first step is the administration of an antibody directed at a tumor-associated antigen that is used to vector a specific enzyme to the tumor site. The enzyme-conjugated antibodies are allowed to localize at the tumor site over a period of time (i.e., 24 h). Next, the tumor-localized enzyme can then convert the subsequently administered prodrug into an active cytotoxic agent in the immediate extracellular fluid surrounding the tumor. The activated drug can then diffuse through the tumor cell membrane or be carried to tumorgenic cells within close proximity that might fail to express the target antigen.

Several ADEPT approaches have been developed and clinically studied thus far, and are reviewed in the literature.[2–5] In particular, alkaline phosphatase conjugated antibodies have been employed to activate phosphorylated prodrugs.[6,7] With this in mind, we considered the use of phosphoramidate prodrugs as possible substrates in an ADEPT or GDEPT approach. Previously, we have shown that amino acid phosphoramidate monoesters of nucleosides are nontoxic compounds that are stable in human sera with $t_{1/2} > 2$ days.[8,9] They have excellent pharmacokinetic parameters as demonstrated in a rat model, with longer half-lives, longer total body clearance rates, and a larger volume of distribution than the parent nucleosides.[9] Furthermore, we have shown that a subset of amino acid phosphoramidate nucleosides are potent antiviral and anticancer agents in their own right with their mechanism of action correlating with the appearance of the corresponding phosphorylated metabolites.[8,10] Previously, it was found that phosphoramidate monoesters of AZT could undergo P–O bond cleavage in the presence of sweet potato acid phosphatase type XA to yield AZT.[11] For ADEPT/GDEPT approaches, a human enzyme is recommended to ensure that the host will not elicit an immumogenic response to the enzyme.

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Consequently, since there are few ADEPT/GDEPT utilizing nuclesides, we chose to examine the potential for nucleoside phosphoramidate monoesters, in combination with human prostatic acid phosphatase (hPAP), to serve as ADEPT/GDEPT nucleoside prodrugs.[2,3]

To test our hypothesis, a series of stable, water soluble aromatic amino acid phosphoramidate monoesters of FUdR and Ara-C were constructed (Fig. 1, **1**–**4**).[8] In an initial experiment to verify that an ADEPT type of approach would work, we used hPAP to cleave the P–O bond of phophoramidates **1**–**4** and examined their antitumor activity based on their ability to inhibit 50% of the cellular growth (GI_{50}) of the human leukemia cell line CCRF-CEM. These values were determined in the presence and absence of hPAP and are given in Table 1. In each case, the cells were treated with the compounds in the presence and absence of hPAP (0.2 unit/mL) for 48 h and the number of remaining viable cells was determined with a trypan blue dye exclusion assay.[12,13]

The GI_{50} values for the Ara-C containing compounds **3** and **4** were similar in CCRF-CEM cells, ranging from 135 to 195 μM, respectively, and thus 1500- to 2167-fold less toxic than the parent nucleoside. However, in the presence of hPAP, **3** and **4** exhibited GI_{50} values of 0.436 and 0.305 μM, respectively. Even though the inhibition was not as potent as was seen with Ara-C, the addition of hPAP increased the potency of **3** and **4** 310- to 640-fold, respectively. The GI_{50} values for the FUdR containing compounds **1** and **2** differed slightly in CCRF-CEM cells, ranging from 0.463 to 4.83 μM, respectively. They were 265- to 2760-fold less toxic than the parent nucleoside FUdR, respectively. However, in the presence of hPAP, **1** and **2** had GI_{50} values of 0.8 and 0.9 nM, respectively. The GI_{50} values of **1** and **2**, in the presence of hPAP, were roughly equipotent to the values that were seen with FUdR in the presence of hPAP, thus increasing the potency of **1** and **2** that were 580- and 5370-fold, respectively.

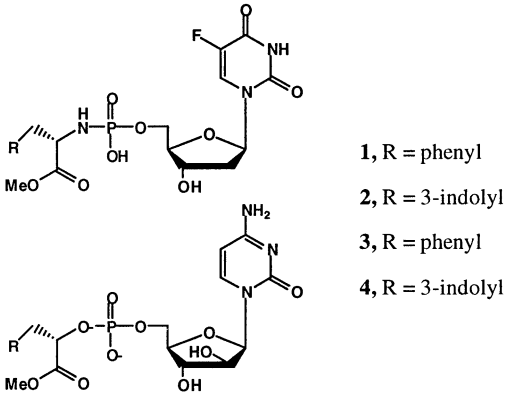

Figure 1. Structures of FUdR and Ara-C prodrugs.

Table 1. In vitro cytotoxicity toward CEM cells of phosphoramidate monoesters and parent nucleosides in the presence and absence of hPAP

Compds	Without hPAP GI_{50} (μM)[a]	With hPAP GI_{50} (μM)[a]	Ratio[b]
FUdR	0.002 (±0.002)	0.0006 (±0.001)	3.33
1	0.463 (±0.05)	0.0008 (±0.001)	578
2	4.83 (±1.8)	0.0009 (±0.002)	5367
Ara-C	0.09 (±0.035)	0.0998 (±0.03)	0.902
3	135 (±6)	0.436 (±0.25)	310
4	195 (±13)	0.305 (±0.11)	639
4 and 0.4 nM **2**	60 (±8)	0.20 (±0.13)	300

GI_{50}, concentration required to inhibit growth by 50% compared to control cultures. The purity of the compounds prior to testing was shown to be >99% by HPLC.
[a]Values are means of three experiments; standard deviation is given in parentheses.
[b]Ratio of GI_{50} without hPAP/GI_{50} with hPAP.

Since the mode of action of Ara-C (DNA chain terminator) and FUdR (thymidylate synthase inhibitor) are different, the possibility of synergy between the phosphoramidates was addressed. Combination treatment with 0.4 nM **2** and varying concentrations of **4** revealed a 3.25-fold increase in the cytotoxicity of **4** in the absence of hPAP and a modest 1.5-fold increase in activity of **4** in the presence of hPAP. Since both **2** and **4** are hPAP substrates, higher concentrations of **4** may interfere with the processing of **2**. This phenomenon would reduce the contribution of **2** to the antitumor activity of the drug combination, irrespective of the mechanisms of action of the nucleoside. Nevertheless, the combination of **2** and **4** in the presence of hPAP was 300-fold more potent than the combination without added hPAP.

Activation of the phosphoramidates **1**–**4** by P–O bond cleavage with hPAP was shown to potently inhibit CEM cell growth. Compounds **1**–**4** are substrates for hPAP, and therefore demonstrate potential as substrates for antibody–hPAP conjugates. Unlike other nucleoside based ADEPT/GDEPT approaches, the broad substrate specificity of hPAP should facilitate the development of a protocol for targeted combination anticancer drug therapy.[2,3]

Acknowledgements

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