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Phospholipid/deoxycytidine analogue prodrugs for the treatment of cancer

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We synthesized thioether phospholipid carrier molecules, conjugated each of them to 1-b-D-arabinofuranosylcytosine (ara-C), and synthesized amido containing phospholipid carriers conjugated to gemcitabine. Changing the alkyl chain at the C1- and C2-positions of the phospholipid increased the conjugates' cytotoxicity over previous conjugates. Dipyridamole increased ara-C's and gemcitabine's IC_{50} value while the IC_{50} values for the phospholipid conjugates were relatively unchanged suggesting that phospholipid conjugates do not require a transporter for entry into the cell. The phospholipid conjugates were cytotoxic to MCF-7 cells and its multidrug resistance-1 (MDR-1) overexpressing cell line derivative (BC-19). Ara-C had no effect on either cell line. Therefore, these novel phospholipid/nucleoside analogue conjugates could be used for the treatment of tumor cells that express certain resistance phenotypes such as a loss of transporter activity and/or MDR-1 overexpression. In vivo *the gemcitabine-phospholipid conjugate was well tolerated and prolonged the survival of tumor bearing mice compared to control mice.*

Key words: Cytarabine – Phospholipid – Conjugate – Drug delivery – Resistance – Chemotherapy – Gemcitabine.

Deoxycytidine analogues such as 1-β-D-arbinofuranosylcytosine (ara-C) and 2'2'-difluorodeoxycytidine (gemcitabine) are valuable chemotherapeutic drugs for the treatment of neoplastic disease. Ara-C is effective against leukemias and lymphomas [1, 2] whereas gemcitabine is useful in the treatment of solid tumors including ovarian [3], pancreatic [4], colorectal [5], lung [6, 7], head and neck [8], urothelial [9], breast [10], and renal [11] cancers. The mechanisms for the biological activity of ara-C and gemcitabine are considered to be well known. These nucleoside analogues enter the cell via a nucleoside transporter [12-14]. Once in the cell, the nucleoside analogue is thrice phosphorylated to yield the active triphosphate metabolite [15-18]. The initial phosphorylation of the nucleoside analogue to the monophosphate by deoxycytidine kinase (dCK) is the rate limiting step in the activation mechanism [13, 19-22]. Once formed, the nucleoside analogue triphosphates are incorporated into DNA where they can inhibit DNA polymerase-alpha [23-25]. The result is DNA strand breaks, chain termination, and cell death. The efficacy of ara-C therapy is directly correlated to the incorporation of ara-C into DNA, the ara-CTP pool size, and the duration of the metabolite's retention within the tumor cell [26, 27]. In addition, gemcitabine has other mechanisms of action for promoting cell death [18, 28, 29], including inhibition of ribonucleotide reductase that further inhibits DNA synthesis [30].

Ara-C therapy can be influenced by drug-resistant disease due to reduced drug uptake or altered prodrug metabolism [31, 32]. In an effort to bypass these processes, the development of nucleoside analogue conjugates linked to phospholipids continues to be pursued. In the early 1980s, Ryu *et al.* [33] coupled ara-C to a series of naturally occurring phospholipids. Efficacy studies comparing ara-C and these conjugates showed promise both *in vitro* and *in vivo*; however, the oral bioavailability of the conjugates was limited due to the metabolism of the conjugates in the GI tract [33, 34]. Continuing developments in the field showed that synthetic thioether-phospholipids circumvented this problem. Thioether-phospholipid conjugates of the nucleoside analog, azidothymidine (AZT), were synthesized [35] and demonstrated oral bioavailability in human clinical trials [36].

Based on the previous work described, we initiated the study of several novel phospholipid molecules conjugated to ara-C or gemcitabine to identify carrier molecules with improved cytotoxic activity. In earlier work, we synthesized the ara-C conjugate 3 (*Figure 1*), and a structurally similar gemcitabine conjugate [37]. Efficacy studies of these conjugates focused primarily on the gemcitabine conjugate due to its cytotoxic activity in comparison to the parental compound. In an effort to improve the cytotoxic activity of the ara-C conjugate 3, we coupled ara-C to two alternate thioether-phospholipids with different alkyl chain lengths at the C1- and C2-postions of the glycerol backbone. In addition, we conjugated gemcitabine to an amido containing phospholipid and this prodrug proved to be the most potent of the phospholipid/deoxycytidine analogues tested in terms of cytotoxicity. Results from these experiments indicated that these new thio and amido containing phospholipid/dexoxycytidine analogue conjugates were able to bypass two resistance mechanisms (loss of human equilibrative nucleotide transporter 1 (hENT1) and multidrug resistance protein 1/P-gp (MDR-1) overexpression) and were cytotoxic to the breast tumor cell line, MCF-7, while the MCF-7 cells were resistant to ara-C, as observed previously [38]. *In vivo* testing of the amido phospholipid/gemcitabine conjugate 5 showed that the prodrug was orally bioavailable and it was effective against Lewis lung carcinoma xenographs in mice.

I. MATERIALS AND METHODS

1. Reagents and general procedures

All reagents were purchased from Sigma-Aldrich or Fisher Scientific and used directly unless otherwise specified. Tissue culture medium and reagents were purchased from Invitrogen, Life Technologies unless otherwise stated. Ara-C was purchased from Sigma-Aldrich.

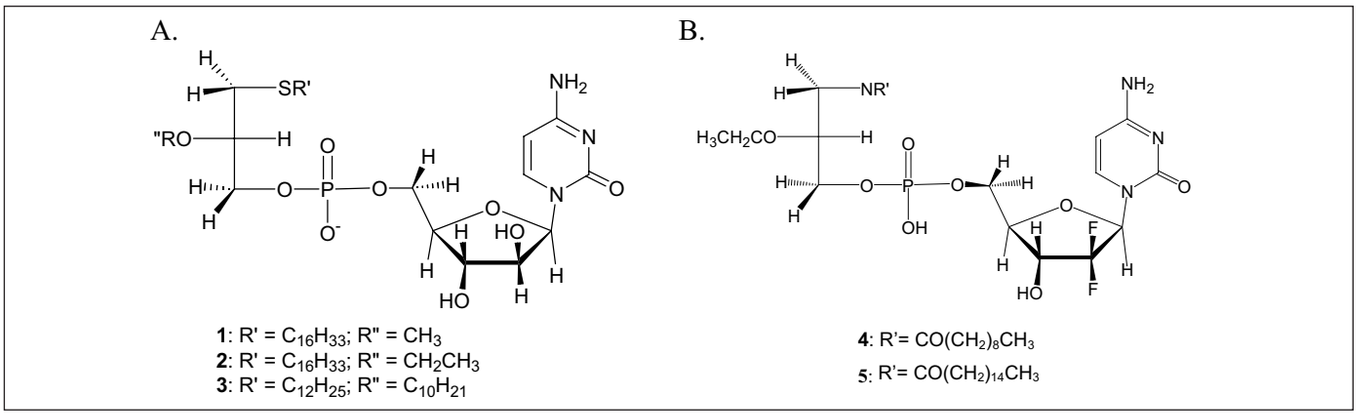

Figure 1 - Chemical structures of the conjugates. Panel A represents those conjugates made with ara-C and panel B represents those conjugates made with gemcitabine.

Gemcitabine was purchased from Leo Chemical Co. (Hong Kong). Phenazine methosulfate (PMS) was purchased from Sigma-Aldrich and 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) was purchased from Promega Corporation (Madison,WI, USA).

2. Synthesis of phospholipid/deoxycytidine conjugates

The deoxycytidine analogue-phospholipid conjugates and all intermediates were synthesized as previously published [33, 37, 39-43]. ^{1}H NMR of the final products was compared to previous results and/or standards, and the final products were subject to high resolution ^{1}H NMR to confirm the synthesis of the desired products 1, 2, 3, 4, and 5 (*Figure 1*).

3. Cell cultures

CEM-SS (human, T-4 lymphoblastoid clone), BG-1 (human, ovarian, adenocarcinoma), HL-60 (human, promyelocytic leukemia), SKLU (human, lung, adenocarcinoma), and Lewis lung carcinoma (mouse, lung) cells were maintained in RPMI-1640 medium supplemented with 10% (v/v) FBS. U373-MG (human, glioblastoma), and SNB 19 (human, glioblastoma), cells were maintained in minimum essential medium supplemented with 10% (v/v) FBS. The human breast cancer cell line, MCF-7, and its stably transfected multidrug resistance derivative cell line, BC-19, were maintained in Dulbecco's modified Eagle's medium (DMEM)/F-12 with 10% (v/v) FBS, and 10 μg/ml insulin. U87 (human, glioblastoma) cells were maintained in DMEM with 10% (v/v) FBS, and SCC-25 (human, tongue, squamous) cells were cultured in the same medium supplemented with 400 ng/ml hydrocortisone. All cells were maintained in log phase growth and kept in a humidified atmosphere of 5% CO_2/95% air at 37°C. All media contained penicillin (100 U/ml) and streptomycin (100 μg/ml).

4. Cytotoxicity and nucleoside transporter inhibition

Cytotoxicity was determined using the CellTiter aqueous non-radioactive cell proliferation assay (Promega Corporation (Madison, WI, USA). Cells were seeded (HL-60, 27,500 cells/well; CEM-SS, 27,500 cells/well; BG-1, 1,500 cells/well; SCC-25, 2,000 cells/well; U373-MG, 3,000 cells/well; Lewis lung carcinoma cells, 1,300 cells/well; SNB 19, 1,700 cells/well; U-87, 1,000 cells/well; SKLU, 1,800 cells/well; MCF-7/WT and BC-19, 2,000 cells/well) 24 h before drug treatment on Costar 96 well culture cluster plates and increasing concentrations from 0.0004 to 100 μM of either ara-C, gemcitabine, or conjugate were added. In some experiments, the nucleoside transporter was inhibited by a 30 min exposure to 20 μM dipyridamole prior to drug treatment [44]. The cells were incubated for 72 h and then a mixture of MTS and PMS was added. The plates were incubated for 4 h, and the results were read on a Precision microplate reader (Molecular Devices, Sunnydale, CA, USA) at 490 nm. Optical densities were compared to the untreated control cells and plotted in GraphPad Prism. Non-linear regression analysis was used to determine the IC_{50} values.

5. *In vivo* experiments

The maximum tolerated dose (MTD) of compound 5 after repeated i.p. injections was determined in NMRI mice (Janvier, Le Genest-Saint-Isle, France). Five mice per dose level (0, 25, 50, and 75 mg/kg/d) were injected (mL/kg) on days 0, 3, 6, and 9. Survival was monitored daily. Body weight was measured on days 0, 3, 8, 14. White blood count, platelet count, hemoglobin, hematocrit, and red blood cell count were measured on days 0, 3, 8, and 13. Bone marrow cell count was measured on day 14. For antitumor efficacy studies, female C57Bl6 mice (Janvier, Le Genest-Saint-Isle, France) were injected with 2.5×10^5 Lewis lung cells (from cell culture) iv/animal/200 μl on day 0. The mice were dosed with either conjugate 5 or gemcitabine as indicated on days 1, 4, 7, and 10. Survival of the mice was measured in days.

II. RESULTS

1. Synthesis and cytotoxicity of the phospholipid/deoxycytidine analogue conjugates

The structures of the intermediates and prodrug conjugates 1, 2, 3, 4 and 5 (*Figure 1*) were confirmed by ^{1}H NMR and/or high resolution mass spectrometry. The resulting spectra were compared to previous results and/or standards to confirm the structures of the intermediate products and the final conjugates.

The cytotoxicity of the conjugates was determined in several different cell lines using the MTS assay. In all cell lines screened, conjugates 1 and 2 had greater cytotoxicity (or a lower IC_{50} value) than conjugate 3 (*Table I*). Although conjugates 1 and 2 demonstrated greater cytotoxic activity than conjugate 3, a direct comparison of ara-C and conjugates 1 and 2 in leukemia cells showed that the conjugates were not as effective as ara-C alone for the incubation times tested. However, both leukemia cell lines tested (HL-60 and CEM-SS) were sensitive to conjugates 1 and 2. More interestingly, ara-C was completely ineffective against the MCF-7 cells (IC_{50} value > 100 μM) while the two conjugates retained measurable cytotoxic activity. Based upon the cytotoxic profile, we abandoned conjugate 3 and focused our efforts on conjugates 1 and 2.

Conjugates 4 and 5 were compared to the cytotoxicity of gemcitabine in several cell lines using the MTS assay (*Table II*). In most cell lines conjugate 5 was equal to or slightly better than gemcitabine in terms of IC_{50}. The IC_{50} values for conjugate 4 were greater than those observed for conjugate 5 for a given cell line. Based on these results, conjugate 5 was selected to undergo *in vivo* testing.

Table I - Summary of ara-C and ara-C-phospholipid conjugates on different cell lines treated for 72 h.

	IC_{50} ± SD, n = 3 (μm)			
Cell line	ara-C	1	2	3
HL-60	0.089±0.012	2.90 ± 0.11	2.57 ± 0.42	86.2 ± 2.7
CEM-SS	0.038±0.006	0.50 ± 0.32	0.38 ± 0.17	12.6 ± 1.2
BG-1	0.33±0.27	13.9 ± 6.3	9.2 ± 0.85	105 ± 43
U373-MG	0.98±0.69	20.5 ± 8.2	33.7 ± 24.2	Not tested
SCC-25	1.35±0.25	21.8 ± 4.3	29.2 ± 5.3	Not tested
MCF-7	> 100	31.6 ± 4.5	29.8 ± 1.9	80.0 ± 6.3

Different cell lines were treated with either ara-C, compound 1, compound 2, or compound 3 for 72 h. Cell viability was measured by the MTS assay. IC_{50} values are reported (mean ± standard deviation).

Table II - A comparison of gemcitabine, conjugate 4 and conjugate 5 in different cell lines.

	IC_{50} (μM)		
Cell line	Gemcitabine	4	5
Lewis Lung	0.02±0.005†	Not tested	0.12±0.058†
SKLU	0.01±0.0001†	0.08±0.038†	0.004±0.004†
MCF7	0.01±0.003†	0.05±0.008†	0.01±0.004†
SNB 19	0.05±0.009*	0.14±0.033*	0.02±0.011*
U 87	0.01±0.004*	0.04±0.010*	0.01±0.003*

Different cell lines were treated with either gemcitabine, compound 4, or compound 5 for 72 h. Cell viability was measured by the MTS assay. IC_{50} values are reported (mean ± standard deviation, †n = 3 or mean ± range* single experiment done in triplicate).

2. Nucleoside transport resistance in HL-60 and CEM-SS cells

Since ara-C is used most commonly for the treatment of leukemia, we investigated the role of the ara-C nucleoside transporter and its effect on the cytotoxicity of conjugates 1 and 2 in HL-60 and CEM-SS cells. Typically, ara-C is transported into the cell through hENT1 which is found in many different cell types [45-47]. To test our hypothesis that the phospholipid/ara-C conjugate may have an advantage over ara-C in resistant tumor cells, we blocked the hENT1 with dipyridamole (20 μM) [44, 45] in the two leukemia cell lines. First, the cells were incubated in the presence or absence of dipyridamole for 30 min prior to drug treatment. Then, different doses of either ara-C, conjugate 1, or conjugate 2 were added to the cells. The results presented in *Figure 2* showed that in the HL-60 cell line (*Figure 2A*), dipyridamole caused a 28-fold increase in resistance with ara-C treatment. Dipyridamole had no effect on conjugate 1 or 2 cytotoxicity. Comparing gemcitabine to conjugate 5, dipyridamole caused a 35-fold increase in IC_{50} values for gemcitabine whereas the IC50 value for conjugate 5 was only 4-fold (data not shown). Using the CEM-SS cell line, the results showed a 140-fold increase in resistance in the presence of dipyridamole and ara-C. These results were in contrast to the 3 to 4-fold resistance observed with conjugates 1 and 2. Taken together, these results suggested that compared to ara-C, conjugates 1 and 2 did not require the hENT1 transporter for entry into the cell and to a lesser extent the same was true for conjugate 5.

3. MDR-1 resistance in the breast cancer cell lines

The initial cytotoxicity profile indicated that the breast cancer cell line (MCF-7) was sensitive to conjugates 1 and 2. One potential problem with conjugating nucleoside analogs to a phospholipid carrier is that they could be a substrate for efflux pumps such as the multidrug resistant transporter MDR-1/P-glycoprotein that remove highly lipophilic drugs from the cytoplasm such as doxorubicin. To test this concern, we utilized the BC-19 cell line, a transfected derivative of the MCF-7 cell line that overexpresses MDR-1 [48]. The results indicated

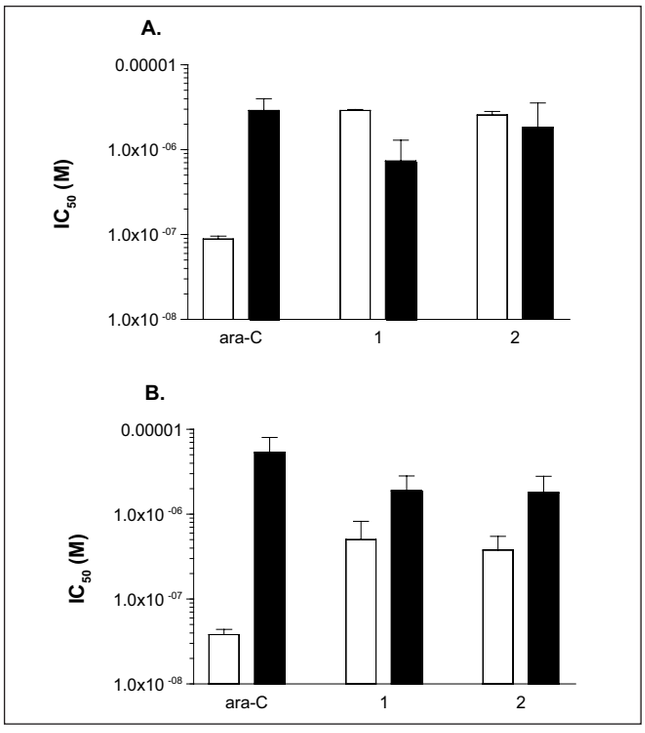

Figure 2 - Leukemia cell lines with nucleoside transporter inhibition. HL-60 (panel A) or CEM-SS (panel B) cells were treated with either ara-C, conjugate 1, or conjugate 2, without (white bars) or with (black bars) dipyridamole for 72 h. The IC_{50} values are plotted on the y-axis. Each bar represents the average (± standard deviation) of three independent experiments.

that in both the MCF-7 and BC-19 cell lines, ara-C was not cytotoxic up to the maximum dose of 100 μM. However, conjugates 1 and 2 were cytotoxic to both cell lines indicating that they were superior to ara-C in these cell lines, and the conjugates were not a substrate for MDR-1 efflux (*Figure 3*). In similar experiments with conjugate 5, there was a modest 5.8-fold difference in the IC_{50} values between the MCF-7 cells and the BC-19 cells (5 ± 0.6 μM versus 29 ± 4.7 μM, respectively). As a positive control, doxorubicin was found to be 20-fold resistant in the BC-19 cells compared to the MCF-7 cells. In summary, synthesizing a more lipophilic prodrug by conjugating nucleoside analogues to phospholipids did not result in a compound that showed a large degree of resistance in cells overexpressing MDR-1 compared to a known substrate for MDR-1, doxorubicin.

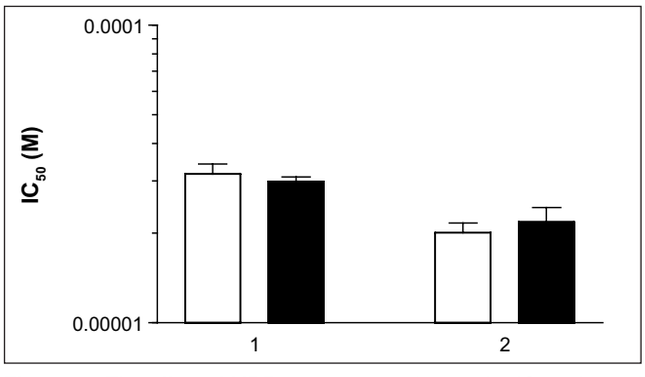

Figure 3 - The effect of MDR-1 overexpression on the IC_{50} values of conjugates 1 and 2. MCF-7 (white bars) or BC-19 (black bars) cells were dosed with either conjugate 1 or 2 for 72 h. Ara-C data not plotted because the cells never reached 50% cell death. Each bar represents the average (± standard deviation) of three independent experiments.

4. *In vivo* treatment of Lewis lung carcinoma bearing mice

The tolerability of compound 5 after repeated i.p. administration was investigated in NMRI mice (doses: 25, 50, and 75 mg/kg/d; treatment: days 0, 3, 6, 9). Survival, body weight and hematological parameters were evaluated for a period of 14 days. The highest dose of 75 mg/kg/d was toxic in terms of mortality and body weight reduction. In addition, hematological parameters and the bone marrow cell count were reduced at this dose. Dosages of 25 and 50 mg/kg/d did not effect survival. Only minor effects (50 mg/kg/d) or no effects (25 mg/kg/d) on body weight and hematology were observed. A mild decrease in bone marrow cell count was observed at both dosages. The MTD for i.p. administration of compound 5 was determined to be 50 mg/kg (Q3 days × 4) and 120 mg/kg (Q3 days × 4) for gemcitabine. On a molar basis this is approximately the same amount of gemcitabine given.

In the Lewis lung tumor model, 50 mg/kg i.p. compound 5 given on days 1, 4, 7, and 10 was equivalent to 120 mg/kg i.p. gemcitabine given on the same schedule ($p = 0.48$) in prolonging survival time compared to the saline control ($p = 0.003$) (*Table III*). It was determined that when conjugate 5 was given orally to mice it had a bioavailability of 34% with a T_{max} = 15 min and a plasma half life of 11 h. In comparison to gemcitabine alone, the i.p pharmacokinetics for gemcitabine given at a dose of 20 mg/kg [49], the T_{max} = 1 min and the plasma half life equaled 17 min. In addition, a dose of 50 mg/kg p.o. conjugate 5 given on days 1, 4, and 7 was equivalent to 50 mg/kg i.p. conjugate 5 given on days 1, 4, 7, and 10 ($p = 0.35$) in prolonging survival time compared to saline control ($p = 0.01$).

III. DISCUSSION

We previously synthesized a phospholipid gemcitabine conjugate and determined the molecule to be cytotoxic to many different cell lines. Furthermore, it could bypass certain resistance mechanisms such as a loss of deoxycytidine kinase, a loss of the nucleoside transporter, and MDR-1 efflux [37, 50]. To determine the effect of different phospholipid carrier molecules on the different phospholipid/deoxycytidine analogue conjugates we investigated the structure activity relationship of the different phospholipid carrier molecules on the cytotoxicity of phospholipid/deoxycytidine analogue conjugates by synthesizing two novel phospholipid carriers that contained a methyl or ethyl ether at the C2- position and a C16 at the C1- position. These carriers were different from the previously synthesized phospholipid carrier that contained a C10 oxy ether at the C2- position and a C12 thio ether at the C1- position. The three ara-C-phospholipid conjugates are shown in *Figure 1*A.

The three ara-C-phospholipid conjugates (1, 2, and 3) were screened for cytotoxicity against different cell lines (*Table I*). The results indicated that conjugates 1 and 2 were more cytotoxic than conjugate 3 in all the cell lines tested, but none were as cytotoxic as ara-C alone. These results demonstrated that the structure of the phospholipid carrier molecule was important when engineering conjugates of small molecular weight drugs. During the screening of conjugates 1 and 2, we made two important observations. First, conjugates 1 and 2 were the most cytotoxic in the leukemia cell lines (CEM-SS and HL-60). This observation was not surprising since ara-C was commonly used as a treatment of hematologic cancers [1, 2]. Second, we found that conjugates 1 and 2 were cytotoxic to the MCF-7 cell line while ara-C alone was not. This result was important since ara-C was known to be ineffective as a cytotoxic agent in the breast cancer cell line, MCF-7 [51, 52]. Taken together, changes in the structure of the phospholipid carrier could alter the pharmacology of known cancer agents and allow them to be more cytotoxic to tumor cells in which they were known to be ineffective.

Conjugation of low molecular weight, water soluble drugs to hydrophobic phospholipids decreases the aqueous solubility and causes the prodrug to favor a more lipid environment. It is reasonable to suggest that these conjugates, as a result of their amphipathic nature, form water soluble lipid aggregates similar in size to large unilamellar vesicles. Using dynamic light scattering [53] we were able to determine that compound 5 in aqueous media formed unimodal spherical particles with a size of 115 ± 2.4 nm. Although we have not explored the exact mechanism of how the phospholipid/deoxycytidine analogue vesicles interact with the cells, it is possible that the entire lipid drug vesicle is taken up by cells in a mechanism analogous to Et-18-O-methyl [54, 55]. It is also possible that monomers of the phospholipid/deoxycytidine analogues at concentrations below the CMC could be interacting with the cell's plasma membrane and enter the cell via passive diffusion. It is unclear at this time how the phospholipid/deoxycytidine analogue conjugates affect the lipid microenvironment of the cells plasma membrane.

One of the important resistance mechanisms that rendered ara-C ineffective was the loss of the nucleoside transporter that transports ara-C across the plasma membrane [50]. Previous reports have indicated that the most important transporter of ara-C was the hENT1 [45-47, 56], and the transport of ara-C via this transporter can be inhibited with compounds such as dipyridamole [44]. We investigated whether or not inhibition of the hENT1 transporter would confer resistance to conjugates 1 and 2 in a manner similar to ara-C by using the leukemia cell lines that were the most sensitive to the two conjugates. Not surprisingly, our results indicated (*Figure 2A and 2B*) that ara-C resistance increased 28- and 140-fold in the HL-60 cells (panel A, white bars) and in the CEM-SS cells (panel B, white bars), respectively. Conjugate 1 and conjugate 2 were unaffected by the inhibition of hENT1 (*Figure 2A*). In the CEM-SS cells (*Figure 2B*), we observed a 3- to 5-fold increase in resistance to conjugates 1 and 2. This slight resistance could be the result of some of the conjugate being metabolized to free ara-C extracellularly and the free ara-C was denied entry into the cell because the nucleoside transporter was inhibited. Clearly, resistance through the inhibition of the hENT1 decreased the cytotoxic activity of ara-C compared to that of the ara-C-phospholipid conjugates. These results support the idea that conjugation of ara-C to a phospholipid carrier could bypass certain resistance mechanisms and allow ara-C to inhibit target cell growth.

One potential problem with these lipophilic conjugates is that they could be substrates for drug efflux pumps that extrude them from the cytoplasm. MDR-1 is a known resistance mechanism that effluxes highly lipophilic compounds such as anthracyclines, taxanes, camptothecins, and vinca alkaloids [57]. To address this issue, we utilized a transfected MCF-7 cell line that overexpressed MDR-1 (BC-19). Conjugates 1 and 2 were equally cytotoxic to both cell lines while ara-C was not cytotoxic at the highest dose tested (*Figure 3*). We conclude from these data that although conjugates 1 and 2 were more lipophilic

Table III - *In vivo* mouse Lewis lung survival with i.p. treatment.

Control 0.9% saline	5 12.5 mg/kg/d	5 25 mg/kg/d	5 37.5 mg/kg/d	5 50 mg/kg/d	Gemcitabine 60 mg/kg/d	Gemcitabine 120 mg/kg/d
18.89 ± 8.937	22.00 ± 3.041	26.56 ± 4.902	29.89 ± 3.655*	34.33 ± 5.339*	30.00 ± 4.213*	34.56 ± 5.457*

Animal survival was monitored after intraperitoneal treatment with compound 5 or gemcitabine on days 1, 4, 7, and 10 after inoculation of Lewis lung carcinoma cells (2.5×10^5 cells iv/animal). As a parameter for tumor efficacy, the mean survival time (days) ± standard deviation of each group and dosage was determined and compared with that of the control group (9 animals were used/treatment group) (*$p < 0.05$).

(*Table I*) they were not a substrate for the MDR-1 efflux pump. These results are of great importance in demonstrating that these two conjugates were superior to ara-C in these cell lines and they were not a substrate for a well characterized resistance mechanism, MDR-1.

Compound 5 proved to be well tolerated and effective in the Lewis lung mouse model. Both i.p. and p.o. routes of administration were statistically better than control-treated animals. Compared to gemcitabine alone compound 5 was not statistically better in survival time; however, the advantage of compound 5 is that it can be given orally whereas gemcitabine is only effect given i.p. In addition, protracted infusion of gemcitabine is greater than a short bolus administration [58]. In pharmacokinetic studies the $T_{1/2}$ of conjugate 5 given p.o. was almost 40-fold greater than the $T_{1/2}$ for gemcitabine given i.p.

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In conclusion, these results support our hypothesis that conjugating small molecular weight drugs such as ara-C and gemcitabine to phospholipid carriers has the potential to improve the pharmacokinetics of nucleoside analogues and other small molecules as well as to overcome certain drug resistance mechanisms. Our future studies will attempt to optimize the carrier molecule to ultimately create a phospholipid conjugate molecule that will be superior to the parent drug while bypassing resistance phenotypes that are clinically relevant. It remains to be seen what role these novel phospholipid conjugate molecules may play as carrier-mediated anticancer agents and also as nanoparticle-sized liposomes for the delivery of other payload anticancer agents and vectors [59].

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