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Evaluation of tetraether lipid-based liposomal carriers for encapsulation and retention of nucleoside-based drugs

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ABSTRACT

Although liposomal nanoparticles are one of the most versatile class of drug delivery systems, stable liposomal formulation of small neutral drug molecules still constitutes a challenge due to the low drug retention of current lipid membrane technologies. In this study, we evaluate the encapsulation and retention of seven nucleoside analog-based drugs in liposomes made of archaea-inspired tetraether lipids, which are known to enhance packing and membrane robustness compared to conventional bilayer-forming lipids. Liposomes comprised of the pure tetraether lipid generally showed improved retention of drugs (up to 4-fold) compared with liposomes made from a commercially available diacyl lipid. Interestingly, we did not find a significant correlation between the liposomal leakage rates of the molecules with typical parameters used to assess lipophilicity of drugs (such logD or topological polar surface area), suggesting that specific structural elements of the drug molecules can have a dominant effect on leakage from liposomes over general lipophilic character.

Since their discovery, liposomes have been widely investigated as a result of their useful properties for drug delivery applications.[1] Liposomes offer several advantages including biocompatibility, biodegradability, ability to carry small or large drug payloads, and a wide range of physicochemical and biophysical properties that can be easily modified to control their biological characteristics.[2–4] Drug retention in the intraliposomal compartment is key to ensure successful delivery of drugs from liposomal formulations. Lipid composition, therefore, becomes crucial for retaining the encapsulated drug. Typical liposomal formulations of drugs are made of either solid-phase phospholipid bilayers (e.g., hydrogenated soy phosphatidylcholine, HSPC) or fluid-phase phospholipid bilayers (e.g., egg phosphatidylcholine, EPC) mixed with cholesterol in order to increase lipid packing and, therefore, decrease membrane leakage.[5,6] Many different hydrophilic and electrically charged compounds such as doxorubicin, vincristine, amphotericin B and morphine have been successfully encapsulated in liposomes leading to FDA-approved liposomal formulations of these drugs.[7–9] However, liposomal retention of small neutral drugs, such as nucleoside analogs, still remains a challenge because small uncharged molecules diffuse quickly through membranes and, therefore, typically cannot be retained in conventional liposomal formulation.[10]

Previously, we reported the design and synthesis of tetraether lipids (such as GMGTPC-CP, Fig. 1) that mimic many membrane properties found in archaeal organisms.[11] We found that membranes formed from pure synthetic tetraether lipids leaked small ions (e.g., H^+, Na^+, OH^-, or Cl^-) at a rate that was about two orders of magnitude slower than liposomes comprised of common bilayer-forming diacyl lipids. In addition, a tetraether liposomal formulation also demonstrated high retention for three different encapsulated drugs commonly used in chemotherapy.[12] In particular, we showed that liposomes comprised of a pure tetraether lipid exhibited a decrease in the rate of leakage of the neutral drug Cytarabine compared to liposomes formed from a commercial diacyl lipid.

Cytarabine is a pyrimidine nucleoside analog antimetabolite that mimics the structure of metabolic pyrimidines. Nucleoside-based molecules are cytotoxic drugs that primarily alter DNA synthesis and replication, and have proven to be useful for the treatment of cancer and viral diseases.[13–16] In order to improve their therapeutic index, several strategies have been evaluated to encapsulate nucleoside analogs into liposomes.[17–19] However, a systematic study on the encapsulation and leakage of other nucleoside analogs from liposomes made from tetraether lipids has not been reported.

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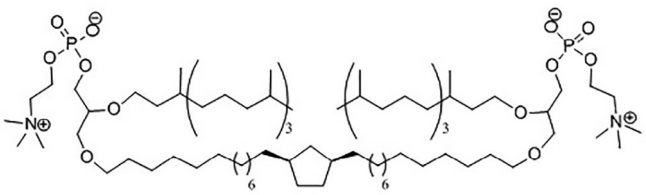

Fig. 1. Chemical structure of tetraether lipid GMGTPC-CP.

Herein, we probe the effects of drug structure on leakage from liposomes made of pure tetraether lipids using a series of 7 nucleoside analogs, which were selected based on small differences in their chemical structure (Fig. 2A). We further restricted this study to molecules that are neutrally charged at physiological pH and have known and useful biological activities as drugs. Specifically, these molecules are pyrimidone-based nucleoside analogues with different sugar derivatives bonded to the base via β-N_1-glycosidic bonds. We hypothesized that their incremental differences in structure could help identify chemical elements that are important for good liposomal retention in this class of compounds. All seven drugs were encapsulated in liposomes made with pure synthetic GMGTPC-CP tetraether lipids which were readily prepared in sufficient quantities to carry out drug leakage experiments (Fig. 1).[11] To examine how encapsulation and retention of the drugs in liposomes made of tetraether lipids differ from liposomes comprised of standard bilayer-forming diacylphospholipids, we also examined leakage of the drugs from liposomes made of 1-palmitoyl-2-oleoyl-sn-glycerol phosphatidylcholine (POPC) lipids (Fig. S1, see supporting information).

Passive encapsulation of all drugs in liposomes made of tetraether and diacyl lipids was achieved as previously reported.[20] Briefly, thin lipid films were formed on glass and hydrated with the drug solution prepared in HBS buffer (pH 7.4). Freeze/thaw cycles and subsequent extrusion with 100 nm and 50 nm polycarbonate membranes provided homogenous distribution of liposomes size, with average radius of ~40–45 nm as estimated by dynamic light scattering measurements (Fig. S2 and Table S1, see supporting information). Free drug was then removed using Sephadex-G25 and lipid concentration was measured using the Bartlett assay.[21] The concentration of the drug encapsulated in the liposome membranes was determined by HPLC. The drug retention was measured over a period of 48 h at 37 °C using a previously reported dialysis assay.[25] For each time point, drug concentration was determined by HPLC and quantified by comparison to a standard calibration curve (see supporting information for details). As a control, the same assay was also used to determine the leakage kinetics of the free drugs across the dialysis membrane in the absence of liposomes (Fig. S3, see supporting information).

Fig. 2 depicts the leakage profiles of 5 of the drugs encapsulated in liposomes formed with either synthetic tetraether lipid (Fig. 2B) or diacyl lipid (Fig. 2C). These five nucleosides exhibited slower diffusion kinetics when they were encapsulated in both types of lipid formulations compared to the dialysis diffusion in the absence of the liposomes (Fig. S3, see supporting information). The remaining two nucleosides, Lamivudine and Zebularine, exhibited rapid diffusion across the dialysis membrane, with or without encapsulation

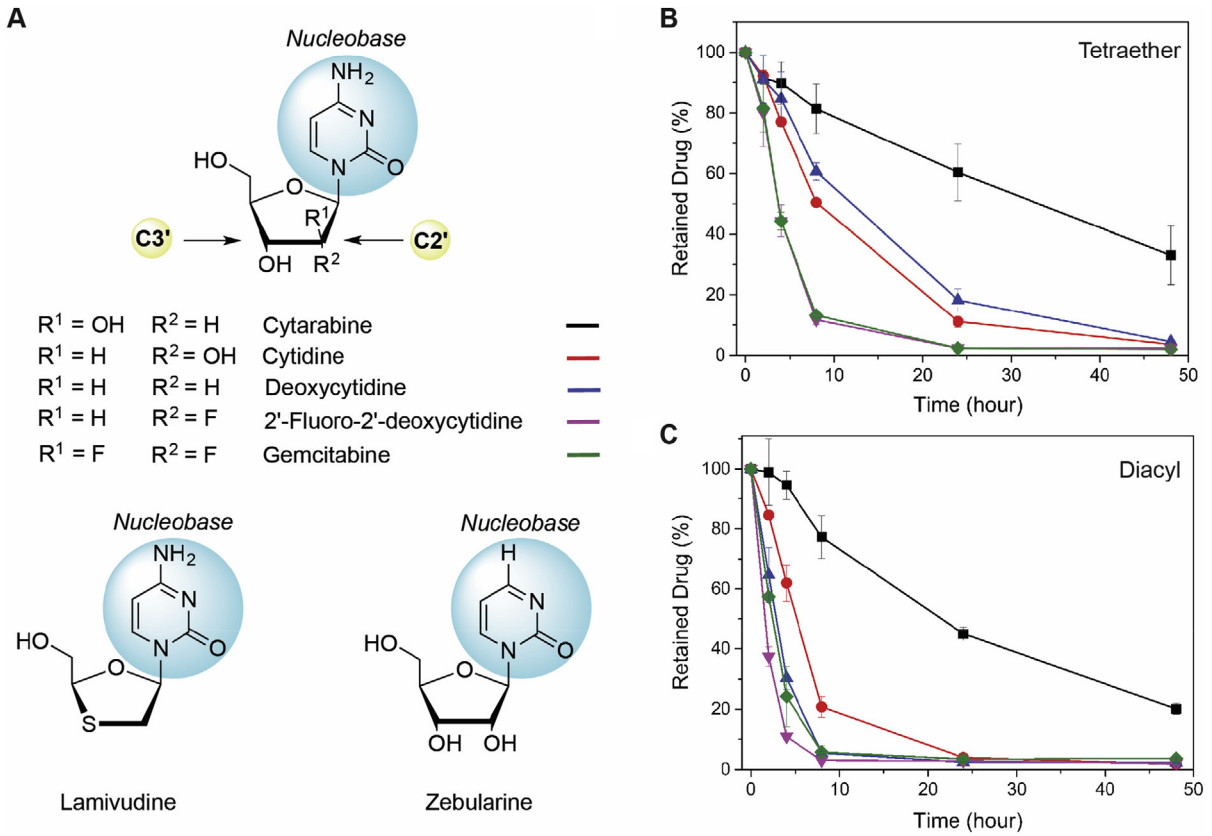

Fig. 2. Chemical structures of the nucleoside drugs (A) and leakage profiles of the drugs studied in this work. Leakage profiles of Cytarabine (black lines), Cytidine (red lines), Deoxycytidine (blue lines), 2′-Fluoro-2′-deoxycytidine (pink lines), Gemcitabine (green lines) encapsulated in liposomes formed with tetraether lipids (B) and diacyl lipids (C). *Leakage profiles of Zebularine and Lamivudine in Graph B and Graph C are not shown due to the observed fast leakage in the dialysis assay used to assess retention of drugs. All measurements were recorded in triplicate.

Table 1
Chemical properties of drug molecules and corresponding observed leakage rate constants (k_{obs}).

	MW (g/mol)	Exp logD[a]	tPSA (Å^2)	SASA (Å^2)	SA (Å^2)	k_{obs} Tetraether Lipid[b] (h^{-1})	k_{obs} Diacyl Lipid[b] (h^{-1})
Cytarabine	243.2	−2.9	562	3055	798	0.017 ± 0.006	0.032 ± 0.008
Deoxycytidine	227.2	−1.9	533	2948	769	0.06 ± 0.01	0.29 ± 0.04
Cytidine	243.2	−2.2	563	3055	798	0.08 ± 0.01	0.15 ± 0.03
2′-Fluoro-2′-deoxycytidine	245.2	−1.8	561	2966	778	0.21 ± 0.04	0.52 ± 0.03
Gemcitabine	262.2	−1.4	588	2985	787	0.20 ± 0.04	0.36 ± 0.05
Lamivudine	229.2	−0.9	509	2679	708	nd	nd
Zebularine	228.2	−2.5	532	2861	750	nd	nd

MW = molecular weight; logD = logarithmic distribution coefficient; tPSA = total polar surface area; SASA= solvent accessible surface area; SA = surface area; nd: not detectable due to fast leakage. tPSA, SASA, and SA were estimated using PyMOL software.
[a] The Exp logD was experimentally determined using a traditional shake-flask method.
[b] The values for k_{obs} are represented as a mean ± SEM (n = 3).

of the drugs in liposomes. Additionally, for the 5 encapsulated drugs, both lipid formulations revealed different leakage profiles according to the structure of the drugs, but liposomes made of tetraether lipids generally showed better retention (up to 4-fold improvement) for the drugs compared with liposomes made from standard bilayer-forming POPC lipids. For instance, Cytarabine exhibited the slowest leakage rates over 48 h, with 40% and 20% of the drug retained in the liposomes made from tetraether or POPC lipids, respectively. The results suggest that chemical groups on position C2′ of the sugar moiety are clearly a driving force for leakage. Removal (as in Deoxycytidine) or change of stereochemistry (as in Cytidine) of the hydroxyl group at this position resulted in faster leakage rates compared to Cytarabine. Even faster leakage of 2′-Fluoro-2′-deoxycytidine and Gemcitabine further supported the importance of a hydroxyl group on position C2′ of the sugar for retention in liposomes. Introduction of one atom (as in 2′-Fluoro-2′-deoxycytidine) or two atoms (as in Gemcitabine) of fluorine on position C2′ led, in both cases, to higher membrane permeability of these compounds compared to Cytarabine, with complete leakage reached within 24 h. Finally, changes in the pyrimidone moiety or changing the methylene to a sulfur atom at position C3′ of the sugar also had significant effects on leakage. In the case of Lamivudine, liposomal encapsulated drugs showed similar leakage in the dialysis assay as the liposome-free drugs, indicating rapid release of this molecule from the liposomes (Fig. S3, see supporting information). Removal of the amino group in the pyrimidone moiety (as in Zebularine) also resulted in nearly complete leakage of the drug during liposome preparation, suggesting that the structure of both the sugar and the nucleobase are important for retention of this class of drugs in liposomes.

In order to evaluate whether the observed leakage of the drugs from liposomes correlated with typical parameters used to assess lipophilicity, leakage profiles were fitted with a mono- exponential function to obtain observed rate constants (k_{obs}, Table 1). These k_{obs} values were then compared to traditional surface parameters that are generally used to characterize lipophilicity of drug molecules such as topological polar surface area (tPSA), solvent accessible surface area (SASA), and surface area (SA) (Table 1). Surprisingly, we did not find a significant correlation between the leakage rate constants and these surface parameters (Pearson correlation coefficient: $r = 0.56$, $p = 0.32$ for tPSA, $r = -0.51$, $p = 0.38$ for SASA, and $r = -0.30$, $p = 0.63$ for SA) (Fig. S4, see supporting information). Furthermore, the distribution coefficient, logD, for all nucleoside drugs was determined experimentally using a traditional shake-flask method.[22] The experimental logD was then plotted against the leakage rates for the drugs examined (Fig. S4, see supporting information). Again, we did not find a significant correlation between k_{obs} values and logD ($r = 0.83$, $p = 0.08$). The logD value for drugs is generally considered a central experimental parameter to predict lipophilicity[23] and, therefore, has been used to predict leakage properties of small organic molecules from liposomes.[24–26] Our results suggest that specific chemical features in the sugar and in the nucleobase can play a dominant role over general lipophilic properties in determining membrane permeability for this class of compounds.

In conclusion, it was confirmed that the use of archaea-inspired tetraether lipids reduces membrane permeability of neutral nucleoside analogs compared to a commercially available diacyl lipid. This result could be attributed to the higher degree of packing offered by tetraether lipids.[10] In addition, molecular parameters typically used to assess lipophilicity (tPSA, SASA, and SA) were not sufficient to predict the relative membrane permeability of the nucleoside-based drugs. This work, thus, reveals that certain chemical features of pyrimidone-based drugs such as fluorine atoms and hydrogen bond donor groups can strongly influence membrane permeability. It remains to be seen whether liposomal retention of other classes of drugs will also be heavily influenced by specific chemical features that can override general lipophilic properties.

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2017.08.032.

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