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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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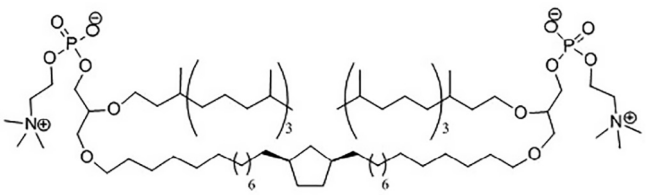

Fig. 1. Chemical structure of tetraether lipid GMGTPC-CP.

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

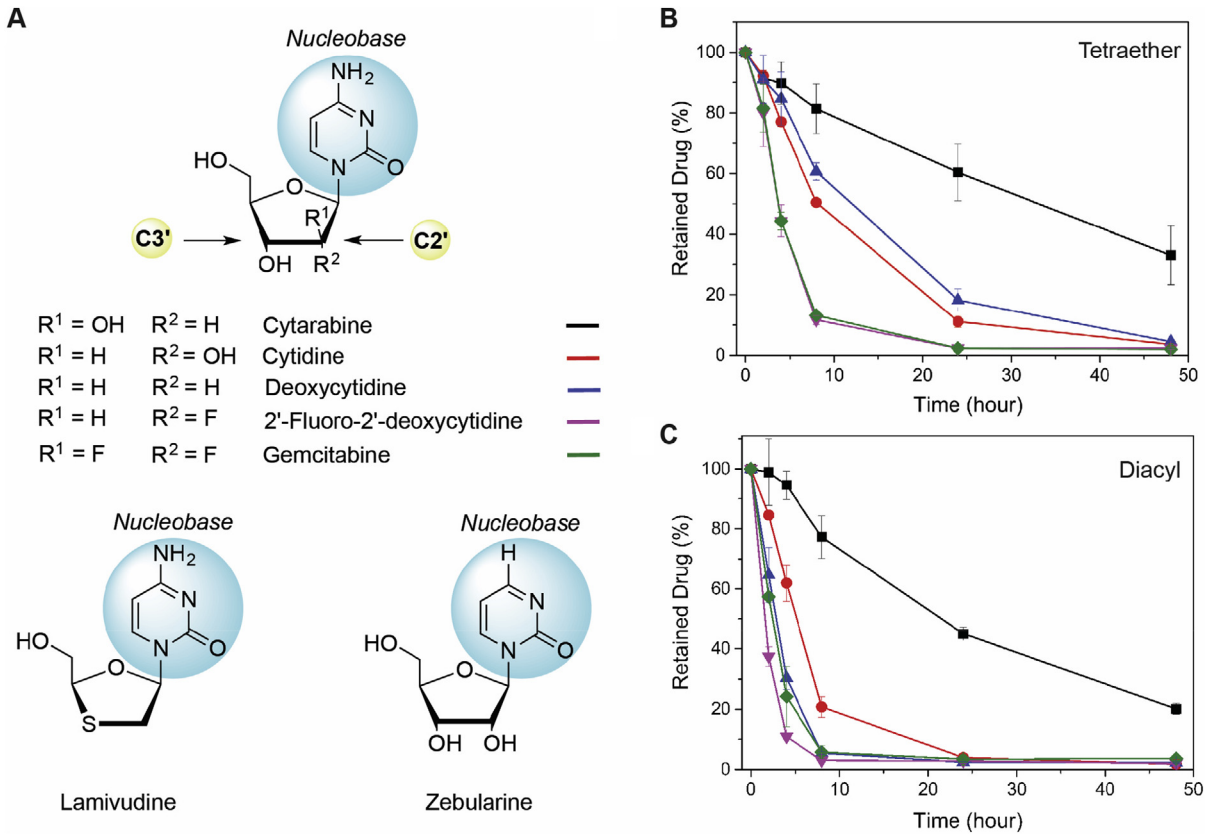

Fig. 2. Chemical structures of the nucleoside drugs (A) and leakage profiles of the drugs studied in this work. Leakage profiles of Cytarabine (black lines), Cytidine (red lines), Deoxycytidine (blue lines), 2′-Fluoro-2′-deoxycytidine (pink lines), Gemcitabine (green lines) encapsulated in liposomes 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.