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Stabilized porous liposomes with encapsulated Gd-labeled dextran as highly efficient MRI contrast agents

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Abstract

A highly efficient contrast agent for magnetic resonance imaging was developed by encapsulating gadolinium within a stabilized porous liposome. The highly porous membrane leads to a high relaxivity of the encapsulated Gd. The stability of the liposome was improved by forming a polymer network within the bilayer membrane.

Magnetic resonance imaging (MRI) procedures have become common practice in diagnostic clinical medicine, due to their ability to acquire high-resolution three-dimensional images of soft tissue and the absence of ionizing radiation. Many of these diagnostic procedures utilize intravenous MR contrast agents, such as gadolinium (Gd), to improve tissue contrast by shortening the longitudinal relaxation time (T1) of surrounding water protons.^{1, 2} Most Gd-based MR contrast agents are small, non-targeted compounds that passively distribute into the intravascular and interstitial space with broad non-specific biodistribution.² In order to compensate for the low signal enhancement generated by individual Gd ions, most targeted Gd compounds have relied on the development of nanoplateforms that can carry a high payload of chelated Gd and which exhibit high longitudinal relaxivities (r1).^{3–10} Currently, a wide range of nanoparticulate platforms including dendrimers,¹¹ liposomes,¹² polymersomes,^{13, 14} micelles,¹⁵ emulsions,¹⁶ and silica nanoparticles,¹⁶ have been tested as platforms for Gd labeling. For these MR contrast agents, it is not only the relaxivity per Gd that defines the effectiveness of the contrast agent but also the number of chelated Gd per nanoparticle. These two parameters can be represented as the relaxivity per nanoparticle.¹⁷

Among the many nanoparticulate systems, phospholipid liposomes are particularly attractive due to the recognized and tested biocompatibility of many phospholipids as well as the increased functionality imparted by their amphiphilic structure. Specifically, hydrophilic drugs can be loaded in the aqueous lumen of the nanovesicles, whereas the hydrophobic domain serves as a natural carrier environment for hydrophobic drugs and the exterior surface can be functionalized with molecularly specific targeting ligands. Liposome-based drugs, Doxil (Janssen Biotech) have already been approved by the US Food and Drug Administration (FDA) for cancer treatment.

The ability of chelated Gd to serve as an effective MR contrast agent largely depends on fast exchange rates between the Gd-bound water and the surrounding bulk water. Liposomes have previously been transformed into effective paramagnetic contrast agents by either encapsulating chelated Gd within the aqueous lumen or by immobilizing the chelated Gd on the membrane surface. In general, immobilization of chelated Gd on the surface of the bilayer membrane is the preferred embodiment for liposome-based MR agents, since surface-bound Gd has much better water accessibility than encapsulated Gd.¹⁸ However, the foreseen advantage of loading chelated Gd within the intra-vesicular volume is the ability to achieve much higher Gd payloads. Based on theoretical calculations, up to tens to hundreds of thousand Gd can be encapsulated within a single ~100 nm liposome. Furthermore, this design is also highly motivated by allowing the liposomes to retain an unobstructed outer surface that can be used for the highly efficient attachment of tumor-cancer targeting ligands. Unfortunately, liposomes with encapsulated chelated Gd have not been widely adopted as highly efficient MR contrast agents due to the detrimental effects of the slow water exchange rate through the vesicle bilayer on the relaxivity of encapsulated Gd.¹² Insufficient chemical and temporal stability further hampers in vivo application of liposomes.¹⁹

In this work we have developed a highly efficient MR contrast agent based on stabilized porous phospholipid liposomes with encapsulated Gd-labeled dextran (Scheme 1). Nanometer-sized liposomes were prepared using polymerizable phospholipid bis-SorbPC [1,2'-bis[10-(2',4'-hexadienoyloxy)decanoyl]-*sn*-glycero-3-phosphocholine]. Due to its unique structure, bis-SorbPC liposomes possess an unusual high membrane permeability compared to most other natural or synthetic liposomes,²⁰ resulting in an increase in the T1-weighted signal of the encapsulated Gd. Furthermore, the chemical and physical stability of bis-SorbPC liposomes can be improved by forming a polymer network within the bilayer membrane. To prevent small Gd-chelates (i.e. Gd-DOTA) from leaking through the porous bilayer membranes, Gd-DOTA was attached to large molecular weight dextran prior to encapsulation. As will be seen later, this attachment increased proton relaxivity of Gd-DOTA by slowing the molecular rotation.^{13, 14}

To investigate the permeability of bis-SorbPC liposome to small analytes, such as H⁺, a pH-sensitive fluorescein isothiocyanate (FITC)-dextran (MW 10kDa) was encapsulated into the aqueous interior of bis-sorbPC liposomes. To confirm that FITC-dextran was too large to pass through the pores in the lipid bilayer, bis-sorbPC liposomes with encapsulated FITC-dextran was centrifuged on a Microcon filtering device with a 100 kDa MWCO membrane. No fluorescence was detected in the flow-through, suggesting that the FITC-dextran is retained within bis-sorbPC liposomes (data not shown). For comparison, FITC-dextran was also encapsulated into 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) liposomes, which is expected to have a low membrane permeability. Both liposomal formulations were suspended in phosphate-buffered saline (PBS) with pH values ranging from 5.5 to 8. As seen in Fig. 1, the intensity of FITC fluorescence in the bis-sorbPC liposome sample exhibited clear pH dependence, with fluorescence increasing with pH. In contrast, the FITC-dextran encapsulated DPPC liposomes exhibited little change in fluorescence as pH was increased from 5.5 to 8. This suggests that small analytes, such as protons, are efficient at traversing the bilayer membrane of bis-SorbPC liposomes. This unusually high membrane permeability is likely due to the packing behavior of bis-SorbPC monomers, which contain an ester moiety in the phospholipid tail (Scheme 1).^{21, 22} Therefore, the bis-SorbPC liposomes offer an opportunity to improve the relaxivity of encapsulated Gd due to their porous membrane structure.

To confirm the stability of the polymerized bis-sorbPC liposomes, the Gd-DOTA-dextran encapsulated bis-SorbPC liposomes were cross-linked via free radical polymerization, and

then treated with surfactant Triton X-100 at a 10:1 molar ratio of Triton X-100-to-lipid. For comparison, unpolymerized bis-SorbPC liposomes were also tested under the same condition. Treatment of unpolymerized bis-sorbPC liposomes with Triton X-100 led to complete liposome dissolution (data not shown), as determined by dynamical light scattering (DLS). However, the mean diameter of the chemically cross-linked bis-sorbPC liposomes was essentially unchanged compared with liposomes that had not been treated with Triton X-100 (Fig. 2). The second peak centered at 10 nm can be attributed to Triton X-100 micelles in the same solution. These data indicate that stabilized bis-SorbPC liposomes have been successfully developed. It should also be noted that the stability of bis-SorbPC liposomes could be adjusted by tuning the polymerization time, the molar ratio of lipid-to-initiator and the type of polymerization, which could allow for tuning of the in vivo stability of these liposomes. Furthermore, their pharmacokinetic properties can be controlled by their surface chemistry, such as the incorporation of PEGylated phospholipids.

To assess the paramagnetic properties of the Gd-encapsulated bis-SorbPC liposomes, the amount of Gd within the sample was determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES). The r_1 relaxivity was then calculated as the slope of the curves $1/T_1$ vs. Gd concentration, as shown in Fig 3. T_1 -relaxation times were determined using a Bruker mq60 MR relaxometer operating at 1.41 T (60 MHz) and at 40 °C. For comparison, the r_1 relaxivities of Gd-DOTA-dextran and Gd-DOTA-dextran-DPPC liposomes were also determined. In all cases, Gd-DOTA was conjugated to 10 kDa dextran. Gd-DOTA-dextran encapsulated within the porous bis-SorbPC liposomes had an r_1 of 9.9 $\text{mM}^{-1}\text{s}^{-1}$ per Gd, which was similar to the r_1 of Gd-DOTA-dextran (9.4 $\text{mM}^{-1}\text{s}^{-1}$ per Gd) in bulk water. It should be noted that the large number of chelated Gd, i.e. up to several thousands, per nanoparticle (Gd-DOTA-dextran encapsulated bis-sorbPC liposome) can be obtained due to a large internal liposomal aqueous space. For the comparison, only low number of chelated Gd, i.e. ~ 3 , per nanoparticle (Gd-DOTA-dextran) can be obtained. This indicates that Gd-DOTA-dextran encapsulated within the porous bis-SorbPC liposomes experiences a fast water exchange rate with surrounding bulk water. In contrast, Gd-DOTA-dextran encapsulated within the nonporous DPPC liposomes had an r_1 of only 4 $\text{mM}^{-1}\text{s}^{-1}$ per Gd, which is more than a 2.4-fold lower than the r_1 for Gd-DOTA-dextran encapsulated-porous bis-SorbPC liposomes. Furthermore, the r_1 measured from Gd-DOTA-dextran encapsulated bis-SorbPC liposomes is 2.6-fold higher than the r_1 of clinically used Gd-DOTA (3.9 $\text{mM}^{-1}\text{s}^{-1}$ per Gd).

The ability of Gd-encapsulated bis-SorbPC liposomes to enhance MRI contrast was further evaluated by acquiring T_1 -weighted MR images using a MRI scanner (9.4T). At a given Gd concentration (0.12 mM), Gd-DOTA-dextran encapsulated within bis-SorbPC liposomes exhibited a significant enhancement in MR signal intensity compared with Gd-DOTA-dextran encapsulated DPPC liposomes (Fig. 4). These results further confirm that porous bis-SorbPC liposomes can efficiently increase the relaxivity of encapsulated Gd compared to nonporous liposomes. Further, the polymerized Gd-DOTA-dextran encapsulated bis-SorbPC liposomes showed similar MR enhancement property compared with unpolymerized Gd-DOTA-dextran encapsulated bis-sorbPC liposomes. These results suggest that the polymerization did not alter porous structure, and the membrane of polymerized bis-SorbPC liposome still retained high water permeability. Therefore, these stabilized paramagnetic liposomes show promise for in vivo MR imaging. Because liposomes have a large internal aqueous space and can achieve high Gd payloads, it is expected that the Gd-encapsulated bis-SorbPC liposome can be used as a highly efficient nanoparticle-based contrast agent.

In summary, we have demonstrated that a highly efficient MR contrast agents could be achieved using Gd-encapsulated bis-SorbPC liposomes. Due to the porous membrane, the encapsulated Gd retained a high r_1 relaxivity. The improved stability of bis-sorbPC

liposomes was achieved through polymerization. By conjugating cancer-targeting ligands to their unobstructed outer surface, these contrast agents have the promise for use as targeted molecular imaging agents.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1. Sensitivity of FITC-dextran encapsulated within porous bis-SorbPC liposomes to pH. Comparisons are made to FITC-dextran conjugates encapsulated within non-porous DPPC liposomes. The fluorescence is normalized relative to intensity at pH 5.5.

Fig. 2. Intensity-weighted hydrodynamic diameter measurements of polymerized bis-SorbPC liposomes before (●) and after (□) adding the surfactant Triton X-100.

Fig. 3.

Relaxivity determination for Gd-dextran conjugates encapsulated within porous bis-SorbPC liposomes (A). Comparisons are made to Gd-dextran conjugates encapsulated within non-porous DPPC liposomes (B).

Fig. 4.

T1-weighted images of Gd-DOTA-dextran-encapsulated liposomes. (1) H₂O; (2) Gd-DOTA-dextran-encapsulated DPPC liposomes; (3) Gd-DOTA-dextran-encapsulated and unpolymerized bis-SorbPC liposomes; (4) Gd-DOTA-dextran-encapsulated and polymerized bis-SorbPC liposomes. Gd concentration was 0.12 mM in all Gd-contained samples.

Scheme 1.

Schematic diagram of stabilized porous bis-SorbPC liposomes with encapsulated Gd-labeled dextran.