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Multi-functional liposomes having temperature-triggered release and magnetic resonance imaging for tumor-specific chemotherapy

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ABSTRACT

For development of tumor-specific chemotherapy, we designed liposomes with temperature-triggered drug release and magnetic resonance imaging (MRI) functions. We prepared multi-functional liposomes by incorporating thermosensitive poly(2-ethoxy(ethoxyethyl)vinyl ether) chains with a lower critical solution temperatures around 40 °C and polyamidoamine G3 dendron-based lipids having Gd^{3+} chelate residues into pegylated liposomes. These stable doxorubicin (DOX)-loaded liposomes retained DOX in their interior below physiological temperature but released DOX immediately at temperatures greater than 40 °C. They exhibited excellent ability to shorten the longitudinal proton relaxation time. When administered intravenously into colon 26 tumor-bearing mice, accumulated liposomes in tumors increased with time, reaching a constant level 8 h after administration by following T_1-weighted MRI signal intensity in tumors. Liposome size affected the liposome accumulation efficiency in tumors: liposomes of about 100 nm diameter were accumulated more efficiently than those with about 50 nm diameter. Tumor size also affected accumulation: more efficient accumulation occurred in larger tumors. Tumor growth was strongly suppressed when liposomes loaded with DOX were administered intravenously into tumor-bearing mice and the tumor was heated mildly at 44 °C for 10 min at 8 h after administration. Multi-functional liposomes having temperature-triggered drug release and MRI functions might engender personalized chemotherapy, providing efficient patient-optimized chemotherapy.

1. Introduction

For establishment of safe and effective tumor therapy, accurate drug delivery is a promising approach that enables specifically targeted attack of malignant cells with drugs [1–3]. Improvement of the tumor-specificity of drug delivery has relied on efficient strategies such as drug carriers: nano-sized particles with long circulation properties with stimulus-sensitive properties. The former can improve accumulation of the carriers in tumor tissues through the enhanced permeation and retention (EPR) effect [4,5]; the latter can increase therapeutic effects by releasing drugs specifically at diseased tissues upon application of stimuli to the target sites [6–8]. Another important function for the improvement of accuracy of drug delivery might be imaging functions of carriers [9–14]. Efficient cancer chemotherapy can be achieved by following accumulation of carriers to the target sites and triggering drugs by application of stimuli after the maximum acculturation of the carriers at the targets sites if drug carriers have these functions and properties. In addition, accumulation processes of the carriers vary among individual patients. Therefore, carriers with these functions can be expected to engender personalized chemotherapy: therapy optimized to individual patients.

Recently, efforts have been made to provide imaging functions to carriers of various types by incorporating various imaging probes such as quantum dots [15–17], magnetic iron oxide nanoparticles [11,12,18], and radionuclide ^{18}F [19,20], which have been incorporated into drug carrier materials used respectively for fluorescence imaging, magnetic resonance imaging (MRI), and positron emission

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tomography (PET) imaging. Among them, MRI is advantageous because it offers high-resolution imaging and no exposure to radiation. In addition, MRI has no time limit for detection in contrast to PET, with ^{18}F whose half-life is 109.8 min. Therefore, MRI is an excellent method for visualization of drug delivery systems in the body from a practical perspective.

Among drug delivery systems of various types, liposomes are of importance as drug delivery vehicles from the viewpoints of biodegradability, size-controllability, and high drug encapsulation ability. Furthermore, stimuli-responsive functions can be provided to liposomes using temperature-induced phase transition of the liposome membranes [21–24] or incorporating temperature-sensitive polymers into the liposome membrane [25–31].

Based on these advantages of liposomes, numerous attempts have been made to provide MR imaging functions to them [14]. Considering that the liposome lumen must be used to encapsulate large amounts of drug molecules, the membrane moiety might be suitable for the incorporation of MRI probes. Therefore, lipid-based MRI contrast agents such as amphiphilic Gd-DTPA derivatives [32,33] have been used for this purpose. Because these molecules contain single Gd-chelate moiety per molecule, inclusion of a high content of these amphiphilic Gd-chelate molecules might be necessary in the liposomes to gain high detectability with MRI, which can be expected to affect the performance of the liposomes. In addition, macromolecular Gd-chelates, which have several Gd-chelate residues in the single molecular chain, have been incorporated to liposomes [13,34,35]. However, incorporation of such macromolecular polychelates taking on an extended structure might affect surface properties of the liposomes.

To obtain multi-functional liposomes that exhibit both highly stimuli-responsive drug release and highly detectable MR imaging functions, we designed biocompatible pegylated liposomes having both highly thermosensitive polymers and amphiphilic poly (Gd-chelates) with a compact conformation. For this purpose, in this study, we newly synthesized polyamidoamine (PAMAM) G3 dendron-based lipids having eight Gd^{3+} chelate residues (G3-DL-DOTA-Gd). Because of the highly branched backbone structure with many chain terminals, the dendron moiety taking on a compact conformation can hold many Gd-chelate residues. Based on the highly temperature-responsive liposomes, which were developed recently by modification of pegylated liposomes with thermosensitive poly [2-(2-ethoxy)ethoxyethyl vinyl ether-*block*-octadecyl vinyl ether (EOEOVE-*block*-ODVE)] with a lower critical solution temperature (LCST) around 40 °C [36,37], we incorporated a dendron-based lipid having many Gd-chelates into the poly(EOEOVE-*block*-ODVE)-modified liposomes (Fig. 1). Performance of the liposomes in terms of visualization and tumor-chemotherapy was investigated. Herein, we describe the importance of visualizing the accumulation process of the liposomes at tumor sites and the heat-triggered drug release function of the liposome to achieve highly reliable and efficient chemotherapy.

2. Materials and methods

2.1. Materials

Egg yolk phosphatidylcholine (EYPC) and N-[methoxy (polyethylene glycol) 5000 or 2000]-distearoylphosphatidylethanolamine (PEG-PE) were kindly donated by Nippon Oil and Fats Co. (Tokyo, Japan). Doxorubicin (DOX) was kindly donated by Kyowa Hakko Kirin Co. Ltd. (Tokyo, Japan). 1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid mono(N-hydroxysuccinimidyl ester) (DOTA-NHS) and fluorescamine were provided from Tokyo Kasei Kogyo (Tokyo, Japan). Cholesterol (Chol) was obtained from Sigma (St. Louis, MO.). PAMAM G1 and G3 dendron-based lipids (G1-DL and G3-DL) were synthesized as reported previously [38]. Copoly

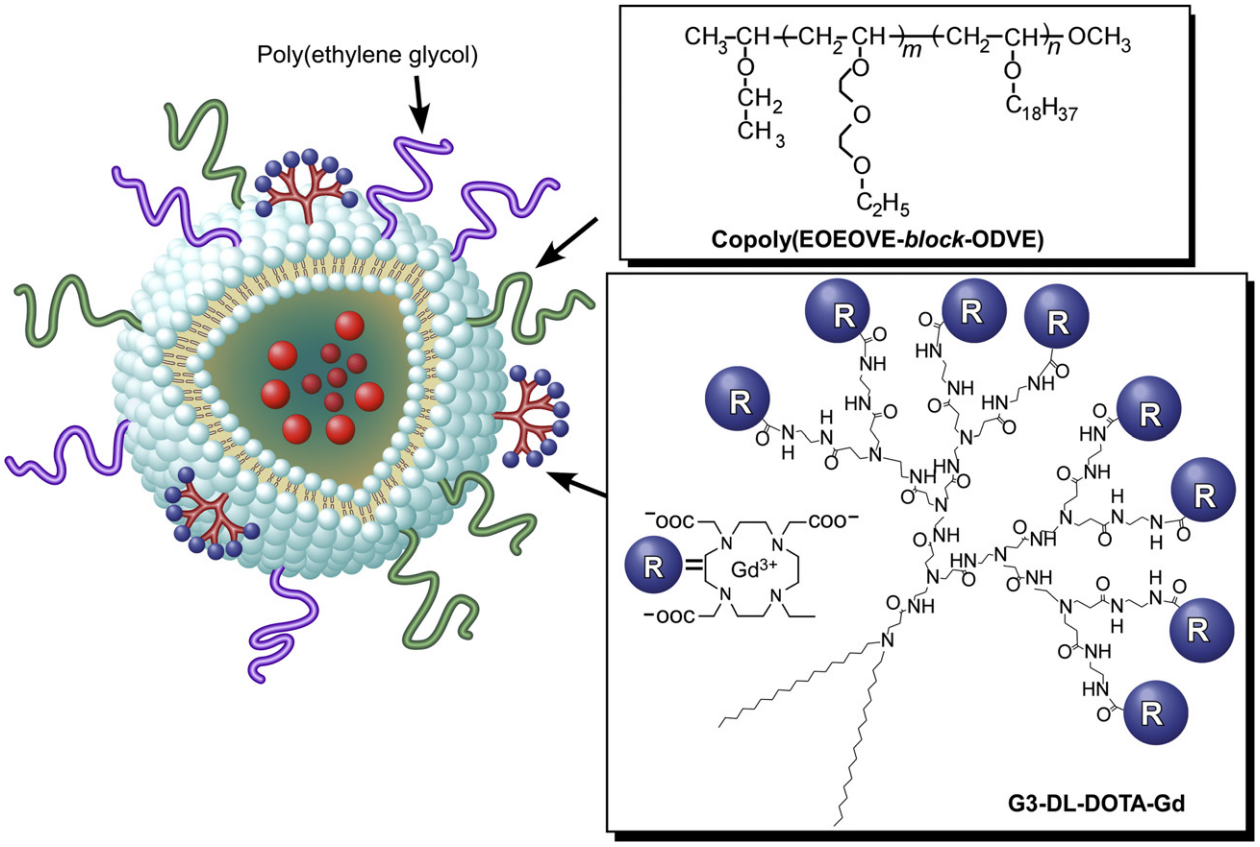

Fig. 1. Schematic illustration of design of functional liposome having temperature-controlled drug release and MR detection functions. Chemical structures of copoly(EOEOVE-*block*-ODVE) and G3-DL-DOTA-Gd were shown in the figure.