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Multifunctional liposomes having target specificity, temperature-triggered release, and near-infrared fluorescence imaging for tumor-specific chemotherapy

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ABSTRACT

We designed functional liposomes with target specificity, temperature-triggered drug release, and near-infrared fluorescence imaging. We prepared the liposomes by triple functionalization of stable pegylated liposomes with thermosensitive poly[2-(2-ethoxy)ethoxyethyl vinyl ether] chains (lower critical solution temperature around 38 °C) with conjugation of antibody trastuzumab (Herceptin, HER), which targets human epidermal growth factor 2, and with incorporation of indocyanine green for near-infrared fluorescence imaging. The liposomes retained DOX in the interior below physiological temperature but released DOX immediately at temperatures higher than 40 °C. The liposomes exhibited excellent ability for association and internalization to target cells overexpressing Her-2, such as SK-OV3 and SB-BR3 cells, and killed these cells when heated at 45 °C for 5 min. When administered intravenously to mice bearing SK-OV3 tumor, the liposomes having HER accumulated in the tumor more efficiently than the liposomes without HER. They stayed there more than 48 h, as judged with near-infrared fluorescence imaging. Furthermore, when the tumor sites of the mice being administered with the DOX-loaded liposomes were heated mildly at 44 °C for 10 min at 7 h after administration, tumor growth was suppressed strongly thereafter. Treatment with the HER-conjugated liposomes produced more efficient tumor-suppressive effects. Results demonstrate that the synergy of target-specific association, temperature-triggered drug release, and imaging is important for efficient tumor chemotherapy.

1. Introduction

For the establishment of safe and effective cancer chemotherapy, numerous efforts have been made to develop high-performance drug carriers that deliver drug molecules specifically to tumor tissues and which kill malignant cells selectively [1–3]. Nano-sized particles with a long circulation have been widely used as drug carriers because such particles efficiently accumulate in tumor tissues through their enhanced permeability and retention (EPR) effects [4,5]. Conjugation of target-specific ligands is a widely used strategy to improve the target-specificity of nanoparticle-mediated drug delivery because such nanoparticles can accumulate in diseased tissues through synergy of the EPR effect and target-specific association with target cells [6,7]. Another important strategy to improve drug delivery accuracy might be stimulus-responsive drug release functions because the release of drugs from such carriers can be triggered specifically at target sites by the application of stimuli at target tissues from the outside of the body [8,9]. Indeed, stimuli of various kinds including temperature, light, ultrasound, and magnetic fields have been used to trigger drug release from nanoparticles [10–14]. Among them, temperature-responsive nanoparticles might be beneficial because hyperthermia has already been used in practical medical applications [15–17].

Temperature-sensitive liposomes are studied intensively for drug delivery systems with stimulus-responsive properties because of their superior biodegradability, drug encapsulation capability, and sharp response to ambient temperature change [18–20]. Temperature-sensitive liposomes were developed first by Yatvin et al. using dipalmitoylphosphatidylcholine-based liposomes, of which the membranes undergo a gel-to-liquid crystalline transition around 42 °C [21]. Thereafter, numerous efforts have been undertaken to produce temperature-sensitive liposomes with higher performance using lysophosphatidylcholine [20], phospholipid DPP-GOG [22], elastin-like peptide [23], and temperature-sensitive polymers [14,18,24–26].

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The efficacy of temperature-sensitive liposomes in anticancer drug-based tumor treatment is well recognized. Especially, temperature-sensitive liposomes prepared using lysophosphatidylcholine have been shown to be effective for tumor-suppression in association with hyperthermia. Those loaded with doxorubicin, which are named "ThermoDox", have reached clinical trials [19,27].

Considering the benefits of temperature-responsive functions and target-specific properties for delivering drugs to target tissues, using a combination of target-specific ligands and temperature-sensitive liposomes might be an efficient strategy to achieve accurate drug delivery because efficient tumor accumulation of the liposomes through synergy of EPR effect and target-specific binding and subsequent drug release from the liposomes upon heating might generate a local drug concentration that is sufficiently high to kill the malignant cells. Obtaining such synergistic effects requires both high stability under physiological conditions and a sharp temperature response upon application of mild heating for the liposomes.

To date, combinations of target-specificity and temperature-sensitivity have been examined using DPPC-based temperature-sensitive liposomes and ligands of various kinds including folate [28], peptides (cRGD, cNGR and CREKA) [23,29,30], antibodies (anti-Her-2 and anti-MUC-1) [31,32] and so on [10,33]. Indeed, these target-specific temperature-sensitive liposomes exhibit higher association to their target cells in vitro. However, improvement of in vivo therapeutic efficacy with the ligand conjugation to temperature-sensitive liposomes has been observed in few cases [23,30]. In addition, local heating has generally been applied before or in an early stage after the liposome administration for in vivo tumor treatment experiments, meaning that the drug molecules might be mostly released from the liposomes before the liposome ligands interact with their receptors of the target cells.

We have developed temperature-sensitive liposomes of different type, which are produced by surface modification of stable liposomes with temperature-sensitive polymers, which destabilize the liposomal membrane above their lower critical solution temperature (LCST) [14,24–26,34]. Especially, poly[2-(2-ethoxy)ethoxyethyl vinyl ether-*block*-octadecyl vinyl ether (EOEOVE-*block*-ODVE)], with LCST around 40 °C, was shown to provide highly temperature-responsive drug release functionality to pegylated liposomes when attached onto the liposome surface [35]. From an earlier study, we observed that intravenous administration of the EOEOVE copolymer-modified pegylated liposomes loaded with doxorubicin (DOX) into tumor-bearing mice and subsequent heating of the tumor at 44–45 °C for 10 min at 6–12 h after the liposome administration caused significant tumor suppression [35].

In addition, an important function for the improvement of accuracy of liposome-based targeted delivery might be the imaging function, which provides information related to the location of liposomes in the body [36,37]. For example, this function enables real-time monitoring of the liposome distribution in the body and reveals liposome accumulation at target sites. The latter information might be especially important to maximize therapeutic effects from temperature-sensitive liposome-mediated chemotherapy because hyperthermia for triggering drug release is applicable at suitable timing when liposome accumulation in the tumor reached the maximum. In fact, we incorporated Gd-chelate-conjugated dendron-lipid into temperature-sensitive EOEOVE copolymer-modified pegylated liposomes for MRI-detection and showed that liposome accumulation at the tumor tissue proceeds 7 h after their administration [36].

Based on our previous study, we expected that conjugation of a target-specific function and imaging functionality to the temperature-sensitive EOEOVE copolymer-modified pegylated liposomes might engender the production of highly efficient drug carriers that accumulate efficiently at tumor tissues through synergy of the EPR effect and ligand-specific interaction and release sufficient amounts of drug molecules instantaneously upon local heating at the best timing, leading to a strong therapeutic effect. Antibody trastuzumab (Herceptin, HER), which targets the human epidermal growth factor receptor 2 (Her-2), was chosen as a target-specific ligand because HER is a widely used antibody with specificity to various types of cancer cells overexpressing Her-2 [39,40]. Additionally, indocyanine green (ICG) was used for near-infrared (NIR) fluorescence imaging, considering that NIR fluorescence imaging with ICG has been used widely for the detection of the biodistribution of liposomes [41,42]. Therefore, for the present study, we attempted to combine the EOEOVE copolymer-modified pegylated liposomes with HER and ICG. Effects of antibody conjugation ICG-incorporation on the performance of EOEOVE copolymer-modified pegylated liposomes were investigated as a drug carrier for cancer chemotherapy (Fig. 1). Benefits of antibody-derived specific interaction of the liposomes to the target cells, detection of liposome accumulation in tumors, and heat-triggered drug release from the liposome to achieve efficient chemotherapy were assessed in this study.

2. Materials and methods

2.1. Materials

Egg yolk phosphatidylcholine (EYPC), N-[methoxy (polyethylene glycol) 5000]-distearoyl phosphatidylethanolamine (PEG-PE) and maleinimide-terminated poly(ethylene glycol) 2000-distearoyl phosphatidylethanolamine (Mal-PEG-PE) were kindly donated by Nippon Oil and Fats Co. (Tokyo, Japan). Doxorubicin hydrochloride salt (DOX) and trastuzumab (Herceptin, HER) were kindly donated

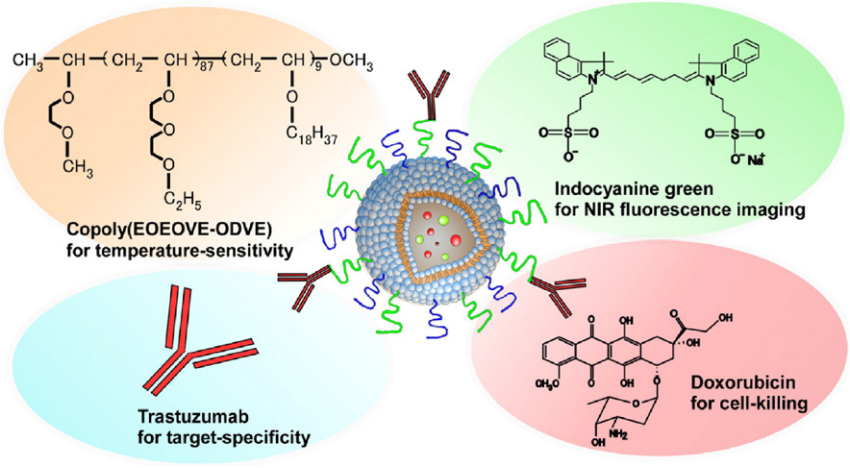

Fig. 1. Design of multifunctional liposomes with target-specificity, temperature-sensitivity and NIR fluorescence imaging for efficient tumor chemotherapy.