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Localized sequence-specific release of a chemopreventive agent and an anticancer drug in a time-controllable manner to enhance therapeutic efficacy

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ABSTRACT

Combination chemotherapy with multiple drugs commonly requires several injections on various schedules, and the probability that the drug molecules reach the diseased tissues at the proper time and effective therapeutic concentrations is very low. This work elucidates an injectable co-delivery system that is based on cationic liposomes that are adsorbed on anionic hollow microspheres (Lipos-HMs) *via* electrostatic interaction, from which the localized sequence-specific release of a chemopreventive agent ($1,25(OH)_2D_3$) and an anticancer drug (doxorubicin; DOX) can be thermally driven in a time-controllable manner by an externally applied high-frequency magnetic field (HFMF). Lipos-HMs can greatly promote the accumulation of reactive oxygen species (ROS) in tumor cells by reducing their cytoplasmic expression of an antioxidant enzyme (superoxide dismutase) by $1,25(OH)_2D_3$, increasing the susceptibility of cancer cells to the cytotoxic action of DOX. In nude mice that bear xenograft tumors, treatment with Lipos-HMs under exposure to HFMF effectively inhibits tumor growth and is the most effective therapeutic intervention among all the investigated. These empirical results demonstrate that the synergistic anticancer effects of sequential release of $1,25(OH)_2D_3$ and DOX from the Lipos-HMs may have potential for maximizing DOX cytotoxicity, supporting more effective cancer treatment.

1. Introduction

Doxorubicin (DOX) is an anticancer drug that has been extensively used to treat a variety of human tumors, including early and advanced breast cancers, by systemic administration [1–3]. However, systemically administered antitumor medication frequently results in rapid clearance of the drug from circulation and poor distribution to the target tissue [4]. Localized delivery of a carrier may concentrate the drug within the tumor, increasing therapeutic effectiveness and reducing systemic toxicity [5,6].

One of the proposed mechanisms of the cytotoxic effect of DOX on tumor cells involves its overproduction of reactive oxygen species (ROS) such as superoxide radicals, which exert irreversible oxidative damage to the cells [7]. The extent of this oxidative cellular damage is determined by the rate of intracellular ROS production and the efficiency of the defense mechanisms of the cells [8,9]. Superoxide dismutase (SOD), an antioxidant enzyme, actively participates in cellular defenses against oxidative damage that is caused by superoxide radicals [10]. Recent studies have suggested that the treatment of tumor cells with 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), a chemopreventive agent, markedly reduces the intracellular expression of SOD, significantly reducing the antioxidant capacity of the cells [11–13]. Therefore, reduction of the cellular expression of SOD by $1,25(OH)_2D_3$ has

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been proposed as a potential strategy for enhancing the cytotoxicity of DOX. The optimal therapy schedule to maximize DOX cytotoxicity in this synergistic action involves pretreating the tumor cells with free $1,25(OH)_2D_3$ and, 24 h later, with the anticancer drug alone.

To provide localized drug delivery with the desired sequence-specific release patterns in a time-controllable manner, this work proposes an injectable, stimuli-responsive co-delivery system that is based on cationic liposomes (Lipos) that are adsorbed on anionic hollow microspheres (HMs) *via* electrostatic interaction (Lipos-HMs, Fig. 1). The cationic Lipos contain $1,25(OH)_2D_3$ and a bubble-generating agent, ammonium bicarbonate (ABC, NH_4HCO_3), and the anionic HMs are fabricated from poly(D,L-lactic-co-glycolic acid) (PLGA). The polymer shell and the aqueous core of each HM contain iron oxide nanoparticles (IONPs) and DOX, respectively. The IONPs that are doped into the PLGA shell can generate heat in response to a non-contact stimulus in a high-frequency magnetic field (HFMF).

Fig. 1 presents the mechanism of localized sequence-specific drug release by the proposed Lipos-HMs system based on the reported optimal schedule [11,14,15]. Upon heating to a temperature of around 41 °C for 10 min by exposure to HFMF, the ABC that is encapsulated in the Lipos rapidly decomposes to form CO_2 bubbles, producing permeable defects in their lipid bilayers, inducing a rapid release of $1,25(OH)_2D_3$ (first-step release), and subsequently generating a high local drug concentration extracellularly. After diffusing into cells, $1,25(OH)_2D_3$ considerably reduces the cytoplasmic level of the antioxidant enzyme SOD, reducing the capacity of the cells to withstand assault by the ROS-generating DOX. When the local temperature exceeds the glass transition temperature (T_g) of PLGA (*ca.* 40 °C), the mobility of the polymer chains is increased, greatly enlarging local voids and thereby releasing DOX from the HMs. Therefore, the entangled PLGA molecules in the HM shell may function as a "molecular switch" that controls drug release. As the heating duration is relatively short (10 min), the extent of release of DOX from the HMs is relatively low.

Twenty-four hours later, the Lipos-HMs are exposed for a longer time (45 min) to HFMF, so the PLGA "molecular switch" in the shells of the HMs is activated for a longer period, greatly increasing the amount of DOX released (second-step release). The ROS-mediated cytotoxicity is therefore enhanced by a reduced level of SOD and a locally high concentration of DOX. Co-delivery of a chemopreventive agent and an antitumor drug in a single carrier system, which can provide on-demand sequence-specific release profiles, not only improves patients' compliance with medication regimens by reducing the frequency of injections, but can also achieves synergistic effects that promote the inhibition of tumor growth. The proposed Lipos-HMs system may be applied to solid tumors with well-defined localization *via* local or endoscopic injection.

2. Materials and methods

2.1. Materials

Poly(vinyl alcohol) (PVA, MW 30–70 kDa), PLGA (with a lactide:glycolide molar ratio of 50:50, MW 24–38 kDa), and $1,25(OH)_2D_3$ were purchased from Sigma-Aldrich (St. Louis, MO, USA), while DOX was obtained from Fisher Scientific (Waltham, MA, USA). Dipalmitoylphosphatidylcholine (DPPC), cholesterol, distearoylphosphatidylethanolamine-polyethylene glycol 2000 (DSPE-PEG2000), and 1,2-di-*O*-octadecenyl-3-trimethylammonium propane (DOTMA) were acquired from Avanti Polar Lipids (Alabaster, AL, USA). All other chemicals and reagents were of analytical grade.

2.2. Preparation and characterization of Lipos-HMs

Lipid film hydration was used to prepare the test Lipos [16,17]. Briefly, a mixture of DPPC, cholesterol, DSPE-PEG2000, and DOTMA at a molar ratio of 60:40:5:5 was dissolved in chloroform. The organic solvent was removed using a rotavapor under reduced pressure until a thin lipid film was formed. This lipid film was hydrated using an aqueous solution that contained ABC (2.7 M) and $1,25(OH)_2D_3$ (0.1 μM). The mixture was then sonicated, before undergoing sequential extrusions at room temperature to control the

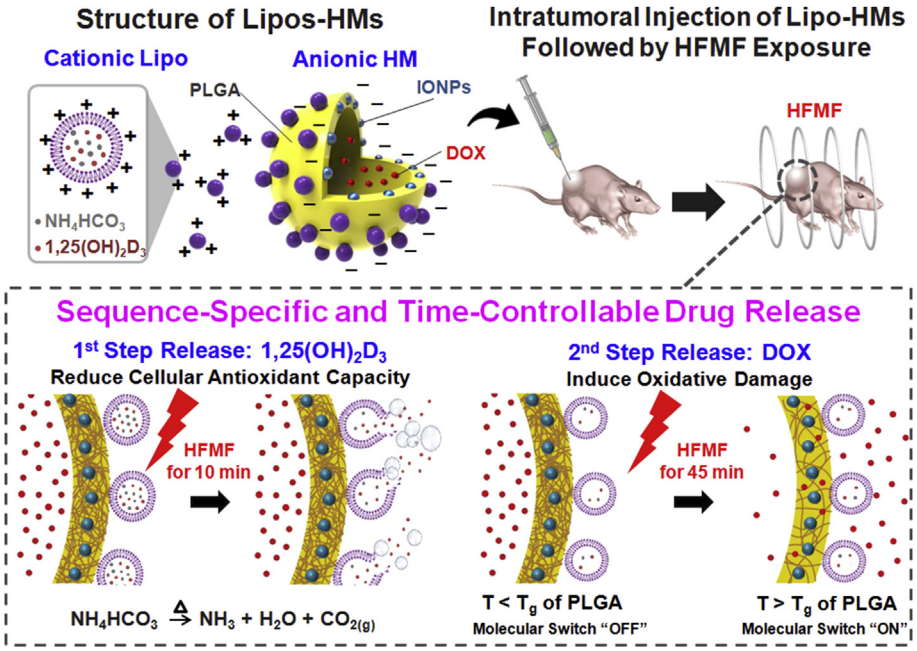

Fig. 1. Structure of Lipos-HMs developed herein and mechanism of their localized sequential release of a chemopreventive agent ($1,25(OH)_2D_3$) and an anticancer drug (DOX) in a time-controllable manner upon application of an external high-frequency magnetic field (HFMF) to enhance therapeutic effectiveness in cancer treatment. Lipos: liposomes; HMs: PLGA hollow microspheres; DOX: doxorubicin.