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Intra-tumor distribution of PEGylated liposome upon repeated injection: No possession by prior dose

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ABSTRACT

Liposomes have proven to be a viable means for the delivery of chemotherapeutic agents to solid tumors. However, significant variability has been detected in their intra-tumor accumulation and distribution, resulting in compromised therapeutic outcomes. We recently examined the intra-tumor accumulation and distribution of weekly sequentially administered oxaliplatin (l-OHP)-containing PEGylated liposomes. In that study, the first and second doses of l-OHP-containing PEGylated liposomes were distributed diversely and broadly within tumor tissues, resulting in a potent anti-tumor efficacy. However, little is known about the mechanism underlying such a diverse and broad liposome distribution. Therefore, in the present study, we investigated the influence of dosage interval on the intra-tumor accumulation and distribution of "empty" PEGylated liposomes. Intra-tumor distribution of sequentially administered "empty" PEGylated liposomes was altered in a dosing interval-dependent manner. In addition, the intra-tumor distribution pattern was closely related to the chronological alteration of tumor blood flow as well as vascular permeability in the growing tumor tissue. These results suggest that the sequential administrations of PEGylated liposomes in well-spaced intervals might allow the distribution to different areas and enhance the total bulk accumulation within tumor tissue, resulting in better therapeutic efficacy of the encapsulated payload. This study may provide useful information for a better design of therapeutic regimens involving multiple administrations of nanocarrier drug delivery systems.

1. Introduction

Liposomes are viewed as one of the most successful nanoplatforms for the delivery of a wide range of therapeutic agents, which includes chemotherapeutic agents [1,2]. Liposomes efficiently potentiate the therapeutic efficacy and significantly alleviate the toxicities of conventional chemotherapeutic agents via altering the pharmacokinetics and bio-distribution patterns of the encapsulated agents following intravenous administration [3,4]. In addition, PEGylated liposomes exert a higher propensity for accumulation into tumor tissue by virtue of their long-circulating characteristics and the inherent leaky nature of tumor vasculature, which potentiates the permeability of liposomes into solid tumors via the so-called "enhanced permeability and retention (EPR) effect" [5–8]. Accordingly, many liposomal formulations of chemotherapeutic agents have been introduced into the market while many others are in line for clinical approval.

However, despite the widespread deployment of liposomal formulations of chemotherapeutic agents in either preclinical animal models or in clinical settings [9–11], their therapeutic efficacy is potentially restrained, at least in part, by the inadequate delivery of efficient concentrations of payload to the tumor tissue. In addition, a growing body of literature has emerged claiming the heterogeneous intra-tumor distribution of PEGylated liposomes following their administration, which can result in a poor therapeutic outcome [12–14]. Such heterogeneous distribution patterns are presumably attributed to the pathophysiological heterogeneity of solid tumors. Furthermore, many studies have emphasized the contribution of the dosage regimen to the intra-tumor accumulation/distribution of PEGylated nanocarriers, and thereby, the overall therapeutic efficacy [15,16].

Recently, we investigated the impact of the dosage regimen on the intra-tumor accumulation of oxaliplatin (l-OHP)-containing PEGylated liposomes and traced the intra-tumor distribution of sequentially administered liposomes in a murine tumor model [15]. We revealed that the dosage regimen substantially affected the tumor accumulation level of administered liposomes. In addition, 3 sequentially administered doses of l-OHP-containing PEGylated liposomes over a 7-day time interval, were distributed more broadly in intra-tumor areas and resulted in potent antitumor efficacy. Such a distribution pattern was presumably attributed to the l-OHP-triggered alteration of the tumor microenvironment via the cytotoxic effect of l-OHP on both the tumor angiogenic vessels and the bulk of the tumor tissue itself. However,

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solid proof of the mechanism underlying such a differential intra-tumor distribution pattern is still lacking.

In the present study, therefore, to exclude the contribution of an encapsulated anticancer drug on the intra-tumor distribution/accumulation of sequentially administered liposomes, we employed "empty" PEGylated liposomes instead and investigated the influence of a dosage regimen on the intra-tumor distribution/accumulation of such sequentially administered "empty" liposomes. In addition, we investigated the contribution of the intrinsic tumor microenvironment, particularly blood flow and vascular permeability, on the fate of PEGylated liposome within the tumor tissue following their sequential administration. The results demonstrated that PEGylated liposome sequentially administered over a shorter time interval of 2-days was distributed at different broader areas within the tumor tissue. This study may provide beneficial information for optimizing the dosage regimen of PEGylated liposomal chemotherapeutic agents.

2. Materials and methods

2.1. Materials

1,2-dioleoyl-sn-glycero-3-phosphoethanol-amine (DOPE), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-n-[methoxy(polyethylene glycol)-2000] ($mPEG_{2000}$-DSPE) and hydrogenated soy phosphatidylcholine (HSPC) were generously provided by NOF (Tokyo, Japan). Cholesterol (CHOL) was purchased from Wako Pure Chemical (Osaka, Japan). O,O′-ditetradecanoyl-N-(α-trimethyl ammonio acetyl) diethanolamine chloride (DC-6-14) was obtained from Sogo Pharmaceutical (Tokyo, Japan). ^{3}H-Cholesterylhexadecyl ether (^{3}H-CHE) was purchased from Perkin Elmer Japan (Yokohama, Japan). 1,1′-dioctadecyl-3,3,3′,3-tetramethyl indocarbocyanine perchlorate (DiI), 1,1′-dioctadecyl-3,3,3′,3-tetramethyl indodicarbocyanine perchlorate (DiD) and 3,3′-dioctadecyloxacarbocyanine perchlorate (DiO) were purchased from Invitrogen (Paisley, UK). All other reagents were of analytical grade.

2.2. Animals and tumor cells

BALB/c mice, (male, 5 weeks old) were purchased from Japan SLC (Shizuoka, Japan). All animal experiments were conducted under the approval of the Animal and Ethics Review Committee of Tokushima University. Colon 26 (C26) murine colorectal carcinoma cells were obtained from the Cell Resource Center for Biomedical Research (Institute of Development, Aging and Cancer, Tohoku University).

The C26-bearing tumor model was established by the subcutaneous inoculation of a C26 cell suspension (2×10^6 cells) into the backs of BALB/c mice. All experiments were initiated when tumors reached 200–300 mm^3 in size.

2.3. Preparation of PEGylated liposomes

Liposomes were prepared by a thin film hydration technique followed by extrusion, as previously described [17]. Briefly, the lipid mixture (20 mM lipids), at the molar ratios shown in Table 1, was dissolved in chloroform and dried under reduced pressure using a rotary evaporator. The dried lipid film was hydrated with 5% dextrose to produce multi-lamellar vesicles. The multi-lamellar vesicles were then sequentially extruded through stacked polycarbonate membranes (Nuclepore, Pleasanton, CA) to yield small uni-lamellar vesicles. In order to trace the liposomes, lipid phase markers (DiI, DiD, DiO or ^{3}H-CHE) were incorporated into the lipid film (1% to total lipid). Liposomal lipid concentrations were determined via colorimetric assay [18]. The particle size and the zeta potential of the extruded liposomes were determined using a Zetasizer Nano ZS (Malvern Instruments, Worcestershire, UK), as mentioned in Table 1.

2.4. Intra-tumor distribution of sequentially administered PEGylated liposomes

The intra-tumor distribution of 2 doses of PEGylated liposomes administered sequentially at different time intervals was evaluated using fluorescently labeled liposomes. Briefly, C26 tumor-bearing mice were initially injected with DiD-labeled PEGylated liposomes (25 mg lipids/kg). After either 24, 48 or 72 h, DiI-labeled PEGylated liposomes (25 mg lipids/kg) were subsequently injected. In addition, DiD-labeled PEGylated liposomes (12.5 mg lipids/kg) and DiI-labeled PEGylated liposomes (12.5 mg lipids/kg) were injected at the same time (0–h interval). At 24 h after the last injection, the mice were euthanized and then tumors were dissected and snap-frozen in OCT compounds with dry-iced acetone. Sections of frozen samples (5 μm thick) were directly examined by fluorescence microscopy (BZ-9000). Three tumors per group were evaluated. The distribution area of each dosed PEGylated liposome and the overlapping area were calculated using Image J software on the basis of the fluorescence emitting area. The overlapping ratio (%) was calculated by dividing the overlapping area by the distribution area of the prior dose PEGylated liposome.

To assess the intra-tumor distribution of each of 3 sequentially administered doses of PEGylated liposomes, C26 tumor-bearing mice were intravenously injected with PEGylated liposomes thrice on day 1 (DiD-labeled PEGylated liposome, 25 mg lipids/kg), day 3 (DiI-labeled one, 25 mg lipids/kg) and day 5 (DiO-labeled one, 25 mg lipids/kg). At 24 h after the last injection, the mice were euthanized and the intra-tumor distributions of sequentially administered fluorescently labeled liposomes were evaluated as described above.

2.5. Intra-tumor accumulation of PEGylated liposomes administered twice at different time intervals

The intra-tumor accumulations of 2 sequentially administered doses of PEGylated liposomes were evaluated using radio-labeled liposomes. C26 tumor-bearing mice were initially injected with non radio-labeled PEGylated liposomes (25 mg lipids/kg). After either 24, 48 or 72 h, ^{3}H-CHE labeled PEGylated liposomes (25 mg lipids/kg) were intravenously injected. At 24 h following the radio-labeled liposome injection, mice were euthanized and tumors were collected. Radioactivity in the tumor tissues was assayed as described previously [19].

2.6. Observation of intra-tumor blood perfusion

Tumor blood perfusion was visualized using a modified double fluorescent dye method [20]. Both DiD-labeled PEGylated cationic liposomes (CL) with a particle size of 350 nm (larger than tumor vascular

Table 1
Physicochemical properties of PEGylated liposomes.

Formulation	Composition (molar ratio)	Particle size (nm)	Zeta potential (mV)
PEGylated neutral liposomes	HSPC/CHOL/$mPEG_{2000}$-DSPE (2/1/0.2)	117.3. ± 11.2	−23.77
PEGylated cationic liposomes	DOPE/CHOL/DC-6-14/$mPEG_{2000}$-DSPE (3/3/4/0.7)	348.9 ± 72.8	+32.04