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Evasion from accelerated blood clearance of nanocarrier named as "Lactosome" induced by excessive administration of Lactosome

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ABSTRACT

Background: Nanoparticle of Lactosome, which is composed of poly(L-lactic acid)-base depsipeptide with diameter of 35 nm, accumulates in solid tumors by the enhanced permeability and retention (EPR) effect. However, a pharmacokinetic alteration of Lactosome was observed when Lactosome was repeatedly administered. This phenomenon is named as the Lactosome accelerated blood clearance (ABC) phenomenon. In this study, the effect of Lactosome dose on the ABC phenomenon was examined and discussed in terms of immune tolerance.
Methods: To tumor transplanted mice, Lactosome (0–350 mg/kg) was administrated. At 7 days after the first administration, indocyanine green (ICG)-labeled Lactosome (ICG-Lactosome, 0–350 mg/kg) was injected. Near-infrared fluorescence imaging was performed, and biodistribution of ICG-Lactosome was evaluated. Further, the produced amounts of anti-Lactosome IgM were determined by enzyme-linked immunosorbent assay (ELISA).
Results: ICG-Lactosome accumulated in the tumor region when the first Lactosome dose exceeded over 150 mg/kg. The amounts of anti-Lactosome IgM were inversely correlated with the first Lactosome doses. Even after establishment of the Lactosome ABC phenomenon with the first Lactosome dose as low as 5.0 mg/kg, the Lactosome ABC phenomenon can be evaded apparently by dosing ICG-Lactosome over 50 mg/kg regardless of anti-Lactosome IgM production.
Conclusions: There are two different mechanisms for evasion from the Lactosome ABC phenomenon before and after its establishment. In either mechanism, however, the Lactosome ABC phenomenon can be evaded by excessive administration of Lactosome.
General significance: Lactosome is a potential nanocarrier for drug and/or imaging agent delivery, which can be used for frequent administrations without significant pharmacokinetic alterations.

1. Introduction

Application of nanoparticles for chemotherapy has been actively investigated on possible control of biodistribution and blood circulation behavior of drugs through their encapsulation into nanoparticles [1,2]. For example, the applications of nanoparticles for the tumor targeted drug delivery of anticancer drugs is actively investigated utilizing phenomenon that nanoparticles are selectively accumulated into solid tumor regions by the enhanced permeability and retention (EPR) effect [3,4].

Lactosome is a polymeric micelle with ca. 35 nm diameter, which is composed of amphiphilic block polydepsipeptide, poly(sarcosine)-*block*-poly(L-lactic acid) [5]. The average block sizes of hydrophilic poly(sarcosine) and hydrophobic poly(L-lactic acid) were 60–90mer and 30mer, respectively. Lactosome shows a long blood circulation behavior and Lactosome labeled with indocyanine green (ICG-Lactosome) successfully imaged tumor orthotopically implanted on liver, which is due to suppression of non-tumor associated capture of Lactosome by liver. The high contrast imaging of tumors in liver becomes possible due to the contribution of hydrophilic poly(sarcosine) chains covering the surface of Lactosome densely in a polymer brush state. However, the biodistribution of Lactosome at the second dose was changed to show a rapid clearance from the blood stream by entrapment in liver [6]. This drastic pharmacokinetic alteration is caused by productions of anti-Lactosome IgM and anti-Lactosome IgG_3 after first administration of Lactosome, because Lactosome induced the T-cell independent (TI) B cell immune response. The similar pharmacokinetic alteration was also observed with PEGylated liposome, which is called as the accelerated blood clearance (ABC) phenomenon [7–10]. The ABC

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phenomenon was also reported on various kinds of polymeric micelles [11,12].

TI antigens like PEGylated liposome can activate B cells and induce IgM antibodies production at the early stage of immunization [13,14]. On the other hand, at high doses, they lack the activation ability on B cells, and immune tolerance against PEGylated liposome was developed [15]. The ABC phenomenon is therefore suppressed by high-dose PEGylated liposome [15–19]. In this study, Lactosome with high dose is examined on the immune tolerance and the Lactosome ABC phenomenon.

2. Materials and methods

2.1. Materials

All regents and solvents were purchased commercially and used without further purifications. Poly(sarcosine)$_{64}$-*block*-poly(L-lactic acid)$_{30}$ (poly(Sar)$_{64}$-*block*-PLLA$_{30}$) and indocyanin green (ICG) labeled PLLA 30mer (ICG-PLLA$_{30}$) were synthesized as previously reported [5,20,21]. For the preparation of Lactosome, poly(Sar)$_{64}$-*block*-PLLA$_{30}$ (1, 5, 10, 15, 25, 35 mg) in test tube was dissolved into chloroform, and then evaporated to form polymer film on the test tube. Water was added to the tube so as concentration of the amphiphilic polymer to be 1 mg/mL (1–10 mg) or 5 mg/mL (15–35 mg). The dispersion was heated at 85 °C for 20 min. After freeze-drying of the solution, saline (1 mL) was added, and the solution was filtered with using syringe filter (pore size: 0.20 μm) just before administration. For the preparation of ICG-Lactosome, ICG-PLLA$_{30}$ (0.5–1 nmol) was additionally mixed into chloroform solution on polymer film formation step. Molar number of ICG-PLLA$_{30}$ was determined using UV absorbance of ICG-Sulfo-OSu at 795 nm. Subsequent protocol is the same with above.

2.2. Instruments

Hydrodynamic diameter of Lactosome was measured by dynamic light scattering (DLS) on a Nano-ZS (Malvern, United Kingdom). Near infrared fluorescence (NIRF) images were taken by Clairvivo OPT (Shimadzu Corp. Japan, ex. 785 nm/em. 845 nm). The pseudo images were constructed from the photon counts. ELISA assay for anti-Lactosome antibody was performed using Multiskan Spectrum Microplate Photometer (Thermo Scientific, USA).

2.3. Preparation of tumor-bearing mice

BALB/c nu/nu mice (Body weight: 20–22 g) were purchased from Japan SLC, Inc. (Japan). SUIT-2/pEF/luc cells (5×10^5 cells) were dissolved in phosphate-buffered saline (PBS, 20 μL), and subcutaneously inoculated into right femoral region of 7-week-old male nude mice [5]. The mice were used for *in vivo* experiments after 2 weeks from the tumor transplantation.

2.4. In vivo near-infrared fluorescence (NIRF) imaging

On experiment, which evaluate effect of first Lactosome dosage amount to the Lactosome ABC phenomenon, tumor bearing mice were divided into 7 groups (n = 3). To the mice of each group, Lactosome of 35 nm in diameter was intravenously injected (5, 25, 50 mg/kg/100 μL, 150, 250, 350 mg/kg/200 μL). To the control mice (Lactosome 0 mg/kg), saline (100 μL) was injected. After 7 days from the first Lactosome dosage, ICG-Lactosome (diameter: 35 nm) was intravenously injected (100 μL, 5 mg/kg) to the mice. Injected ICG amount was set to be 5 nmol/kg. NIRF images were taken at 5 min and 24 h after the administration. During the imaging process, the mice were held on the imaging stage under anesthetized condition with 2.5% of isoflurane gas in air flow (1.5 L/min).

On experiment, which evaluate effect of second Lactosome dosage amount to the Lactosome ABC phenomenon, tumor bearing were divided into 6 groups (n = 3). To the all mice except control group ones, Lactosome of 35 nm in diameter was intravenously injected (100 μL, 5 mg/kg). To the mice of control group (Lactosome 0 mg/kg), saline (100 μL), was injected. At 7 days after the first Lactosome administration, ICG-Lactosome (diameter: 35 nm) was intravenously injected (5, 25, 50 mg/kg/100 μL, 150, 350 mg/kg/200 μL) to the mice. Injected ICG amount was set to be 5 nmol/kg on all groups. NIRF images were taken in the same method with above.

2.5. Preparation of serum

On experiment, which evaluate effect of first Lactosome dosage amount to the Lactosome ABC phenomenon, mice blood was collected after 7 days from the first Lactosome (diameter: 35 nm, injection volume: 100 μL (5, 25, 50 mg/kg), 200 μL (100, 150, 200, 250 mg/kg)) dosage. Collected blood from inferior vena cava under anesthesia condition was transferred into Microstrainer® tube (BD Corp. USA). Blood serum was separated by centrifugation (10 min, 3000 rpm) and saved at −40 °C.

On experiment, which evaluate effect of second Lactosome dosage amount to the Lactosome ABC phenomenon, mice were occurred ABC phenomenon to be injected Lactosome (5 mg/kg). To the mice, Lactosome (diameter: 35 nm, injection volume: 100 μL (5, 25, 50 mg/kg), 200 μL (150, 350 mg/kg)) was injected at 7 days after first injection. The mice blood were collected at 7 days after second administration, and treated in the same way with above.

2.6. Enzyme-linked immunosorbent assay (ELISA) experiment

Lactosome (0.5 μg) in 50 μL distilled water was added to 96-well plates and air dried completely for 1 day. Then, 150 μL of blocking buffer (2% BSA/PBS) was added and incubated for 2 h. The wells were washed four times with washing buffer (PBS-T: 0.05% Tween 20 in PBS). Mice serum were added to the wells and incubated for 2 h. After the incubation, the wells were washed four times using PBS-T. Peroxidase conjugated goat-anti-mouse IgM in 0.1% BSA/PBS (50 μL, Southern Biotech, USA) was added as the secondary antibody. After incubation of the solution for 2 h, and then the wells were washed again four times with PBS-T. *o*-Phenylenediamine (0.5 mg/mL, Sigma, St. Louis, MO), which was dissolved in 0.0003% H_2O_2-0.1 M citrate phosphate buffer (pH 5.0), was added to the microplate. 10 min after the *o*-Phenylenediamine addition, optical density (OD) was determined from UV absorbance at 490/reference at 620 nm.

2.7. Ethics

All of our *in vivo* animal experiments were approved by the Animal Research Committee of Kyoto University. Animals were treated humanely.

3. Results

3.1. Preparation of Lactosome and ICG-Lactosome

Lactosome, which is composed of poly(Sar)$_{64}$-*block*-PLLA$_{30}$ amphiphilic polydepsipepitde, was prepared by the film rehydration method. ICG-Lactosome was prepared by the same method with Lactosome but with an appropriate amount of ICG-labeled poly(L-lactic acid) 30mer (ICG-PLLA$_{30}$) in addition to the amphiphilic polydepsipeptide on polymer film preparation (Fig. 1). Lactosome showed no toxicity up to 2000 mg/kg (Fig. S1) and blood-half time of ICG-Lactosome was calculated to be 17.8 h in mice (Fig. S2).