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Research paper

Gadolinium incorporated reconstituted chylomicron emulsion for potential application in tumor neutron capture therapy

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Abstract

Gadolinium (Gd) neutron capture therapy (NCT) is currently under development as a potential approach for tumor therapy. Nanoparticles have been suggested as a potential delivery system to carry or target Gd to tumors for thermal or epithermal neutron irradiation. The reconstituted chylomicron emulsion is an artificial chylomicron remnant prepared using commercially available natural and biocompatible lipids. We proposed to use this nanometer-scale emulsion to deliver Gd to solid tumors by modifying the surface of the emulsion. A lipophilic Gd compound, gadolinium acetylacetonate (GdAcAc), was incorporated into the emulsion, resulting in a final pure Gd concentration of more than 1 mg/mL. The apparent solubility of GdAcAc was enhanced by about 6000-fold by this incorporation. The emulsion particles were shown to be stable in a two-week short-term stability study when stored at 4 °C. In addition, no extensive particle aggregation was observed when the emulsion particles were incubated in simulated biological media such as serum. Also, GdAcAc does not significantly 'leak' out from the emulsion particles. Only ~5% was released in 20 h in a SDS (0.5% w/v) in phosphate buffered saline (pH 7.4, 10 mM) medium. Finally, the emulsion particles were coated with polyethylene glycol (PEG), and injected into Balb/C mice via the tail vein. A significant proportion (71.6 ± 18.4%) of the PEG-coated, GdAcAc-incorporated emulsion remained circulating in the blood 5 h after the injection, while the PEG-free emulsion was mainly accumulated inside the liver. This chylomicron emulsion may be used to deliver Gd into solid tumors for NCT.

Keywords: Nanoparticles; tumor targeting; PEG; biodistribution; lipoprotein

1. Introduction

Neutron capture therapy (NCT) is a potential biphasic radiotherapy for cancers. A stable, non-radioactive nuclide is first delivered to tumors. Then, upon irradiation of the tumors by external thermal or epithermal neutrons, the nuclide produces localized cytotoxic radiations that can kill tumor cells [1,2]. Earlier studies were mainly focused on using ^{10}B as a nuclide for the treatment of melanoma and brain glioma [3–5]. Gadolinium has been proposed and investigated as an alternative to boron for NCT [6–8]. Upon absorption of neutrons, ^{157}Gd will emit killing radiations such as γ rays and Auger electrons by a $^{157}Gd + n_{th} \rightarrow {}^{158}Gd + \gamma + 7.94$ MeV reaction [9]. ^{157}Gd as a nuclide has many advantages over ^{10}B. First, the thermal neutron capture cross-section of Gd is 255,000 barns, which is about 66-times greater than that of ^{10}B [6]. Therefore, when compared to ^{10}B, ^{157}Gd absorbs neutrons more efficiently and requires shorter irradiation times. Second, Gd-neutron capture reaction leads to emission of photons with tumor killing energy deposition at longer ranges in tissues. When ^{157}Gd absorbs neutron, it releases γ rays followed by a series of low energy conversion and Auger electron. The internal conversion electrons have a range of about 60 mm, compared to 9 mm for boron decay, and thus, are able to kill more tumor cells [10]. Finally, many Gd compounds (e.g. Magnevist®, Berlex Laboratories, Inc.) have been used as contrast agents in magnetic resonance imaging (MRI) [11,12], providing the opportunity for a potential future integration of MRI diagnostics with GdNCT for tumors.

As a pre-requirement, the success of tumor NCT is based on the accumulation of a sufficient amount of Gd in tumor tissues prior to neutron irradiation [9]. Currently, there is not a Gd compound that can selectively target and be retained in tumors. Therefore, drug delivery systems that target Gd to tumors and help enhance its retention are actively sought. In the last decade, Gd compounds have been incorporated into or conjugated on a range of particulates such as calcium carbonate

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microparticles [13], gadopentetic acid-loaded chitosan microparticles [7,14,15], lipid emulsions [16], and nanoparticles prepared with emulsifying wax as the matrix material [8,17]. The use of nanoparticles, especially those with a size less than 400 nm, and ideally around 100 nm, is advantageous because it has been shown that smaller particles target tumors more efficiently than larger ones [18–21].

Lipoproteins are naturally occurring biological emulsions that carry cholesterol and other lipids in the blood circulation [22–24]. Chylomicron is a triglyceride-rich lipoprotein that is formed by packing dietary lipids absorbed through the intestine [25,26]. In the blood circulation, chylomicrons accept apolipoproteins, such as apo E. These modified chylomicron remnants are taken up by liver hepatocytes via apoprotein receptors [27]. Previous studies have shown that the extent of uptake of reconstituted chylomicrons by hepatocytes was dependent on whether a sufficient amount of apo E protein was pre-coated on the surface of the chylomicrons prior to the injection [28]. Apo E protein can bind to chylomicron while in blood circulation [29]. Other apoproteins, such as apo C, that might display an opposite effect on the receptor binding, also compete with apo E in occupying the binding sites on chylomicron emulsions [30,31]. Therefore, we hypothesized that a modification of the surface of the reconstituted chylomicron could enhance its circulation time in blood by preventing the binding of apoproteins, and thus allowing potential passive targeting of the surface modified chylomicron to solid tumors. Due to the highly permeable vasculature of tumors, long-circulating emulsion particles with size around 100 nm can pass through the leaky endothelium tissues of tumors [19,32]. These emulsion particles should also help to enhance the retention of the Gd compound incorporated inside them in tumors [14], if the Gd compound does not significantly 'leak' out from the chylomicron emulsion.

Of the many techniques used to enhance the blood circulation time of particles, surface coating of particles with polyethylene glycol (PEG) polymers has proven to be successful [32–34]. PEG, an amphiphilic polymer, when coated on the surface of particles, can favorably modify the surface hydrophilicity/hydrophobicity of particles and sterically stabilize them, and thus, suppress the binding of serum proteins (e.g. apoproteins) and other opsonic factors [32,34,35]. In addition, some particle-macrophage interactions may also be prevented by the PEG coating [32]. Studies using liposomes and nanoparticles prepared with various matrix materials have shown that 1–7 mol % of PEG with a molecular weight no less than 2000 Da were effective in prolonging their blood circulation time [36–38].

In this present study, we successfully incorporated gadolinium acetylacetonate (GdAcAc), a compound with very limited water solubility, into the reconstituted chylomicron emulsion and characterized it. Surface coating of the GdAcAc-incorporated chylomicron emulsion with PEG resulted in a significant change in the biodistribution profile of the incorporated Gd in mice.

2. Materials and methods

2.1. Materials

Sephadex G-75, olive oil (refined), cholesterol (Chol), cholesteryl oleate (CO), and GdAcAc were purchased from Sigma-Aldrich (St. Louis, MO). L-α-phosphatidylcholine (PC) and lyso-phosphatidyl choline (lyso-PC) were purchased from the Avanti Polar lipids, Inc. (Alabaster, AL).

2.2. Preparation of chylomicron emulsions and incorporation of GdAcAc into them

The emulsion was prepared as previously described [25,28]. Briefly, olive oil, PC, lyso-PC, CO, and Chol were dissolved in chloroform in a glass vial at a weight ratio of 70:22.7:2.3:3.0:2.0. After the chloroform was evaporated, the lipid thin film left was hydrated overnight at 4 °C with filtrated (0.2 μm) and de-ionized water to obtain a final lipid concentration of 40 mg in 1 mL of water. The lipid suspension was vigorously vortexed for 30–60 s, incubated in a 65–67 °C waterbath for 5 min, and then sonicated in a waterbath sonicator (VWR International) for 5 min or until a semi-transparent fluid emulsion was formed. The emulsion was extruded sequentially through 1.0, 0.4, and 0.1 μm filters using an Avanti Mini-Extruder (Avanti Polar Lipids, Inc.).

To incorporate GdAcAc into the emulsion, GdAcAc was co-dissolved with the lipid mixture in chloroform. The dried lipid film was treated similarly as mentioned above. Emulsion cannot be formed if the GdAcAc concentration was over 20% (w/w) of the total lipids used.

To coat PEG on the surface of the emulsion, DSPE-PEG (2000) maleimide (1,2-Distearoyl-sn-Glycero-3-Phosphoethanolamine-N-[Maleimide(Polyethylene Glycol)2000], Ammonium Salt) (Avanti polar lipids) (2.5% to the total lipids, m/m) was co-dissolved with other components of the emulsion in chloroform prior to chloroform evaporation.

2.3. Gel permeation chromatography

To separate GdAcAc-incorporated emulsion particles from unincorporated GdAcAc, the GdAcAc-emulsion (0.1 mL) was carefully applied to a Sephadex G-75 column (diameter = 0.7 cm, length = 25 cm) and eluted with water. The concentration of GdAcAc in each fraction (of 1 mL) was measured using a DU® 640 spectrophotometer (Beckman-Coulter, Fullerton, CA) after the GdAcAc was extracted from each fraction using the Bligh and Dyer monophase (water/chloroform/-methanol, 1:1:2.1, v/v/v) [39]. GdAcAc has a strong absorption at 288 nm. Similar method had been used previously to purify GdAcAc-incorporated solid-lipid nanoparticles [40].

2.4. Particle size determination

The size of the emulsion was determined using a Coulter N4 Plus submicron particle sizer (Beckman Coulter, Inc.) following the procedure recommended by the manufacturer.

Particle size was reported as mean ±S.D. (polydispersity index) of a representative of 2–3 determinations.

2.5. Stability of the emulsion particle in simulated biological media

To predict whether the emulsion particles will extensively aggregate or bind to serum proteins when introduced into the blood circulation, the particle size of the emulsions was measured after they were incubated in phosphate buffered saline (10 mM, pH 7.4) and in 10% (v/v) fetal bovine serum (FBS) in PBS at 37 °C for 30 min [40,41].

2.6. The release of GdAcAc from the emulsion

To monitor the release of GdAcAc from the emulsion, GPC-purified, GdAcAc-incorporated emulsions that contain 1.0 mg of GdAcAc in 1.0 mL of 0.5% SDS (w/v) dissolved into PBS (10 mM, pH 7.4) was placed into a cellulose ester dialysis tube (molecular weight cut, 3.5 kDa) (Spectrum Chemicals and Laboratory Products, New Brunswick, NJ). The tube was placed into 50 mL of 0.5% SDS dissolved into PBS (10 mM, pH 7.4) and incubated in a 37 °C shaker incubator. At 0, 5, 10, 15, 30, 60, 120, and 240 min and 19 h, the dialysis tube was taken out and placed into another 50 mL of fresh release medium. The concentration of GdAcAc in the release medium was determined using a UV spectrometer.

To verify the feasibility of using the dialysis tube to study GdAcAc release, the diffusion of free GdAcAc (dissolved into 0.5% SDS in PBS) through the membrane of the dialysis tube was also investigated.

2.7. Biodistribution of GdAcAc-incorporated emulsions in mouse

Ten to twelve-week-old Balb/C mice were used for the biodistribution study. Purified GdAcAc-incorporated emulsions (containing 250 μg GdAcAc and in 0.2 mL of 5% dextrose) were injected into mice via the tail vein. The mice were lightly anesthetized (i.p.) with pentobarbital (Nembutal® Sodium Solution, Abbott laboratories, North Chicago, IL) prior to the administration, and were sacrificed with CO_2 5 h later. The blood, liver, spleen, heart, lung, and kidney were harvested. The blood was stored at −20 °C prior to further analysis. Other harvested organs were washed with PBS (10 mM, pH 7.4) three times, and incubated in a 60–65 °C oven overnight.

2.8. Measurement of GdAcAc concentration in the sample

The concentration of GdAcAc in each organ and the blood was determined using an Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES Varian Liberty 150, Varian Inc., Palo Alto, CA). Previously described instrument parameters and method validation were utilized [7,8,42,43]. The calibration curves were prepared with a serial dilution of GdAcAc standard solution in nitric acid (3.3 N). Acid digestion of the samples was carried out as previously described with slight modification [44]. Briefly, each dried sample was weighed and placed into a 20 mL glass scintillation vial. Three ml of nitric acid (6.6 N) was added. The vial was tightly closed and kept in a 60 °C oven for 12 h. The vial contents were clear, yellow solutions after the digestion. After cooling to room temperature, each sample was diluted with de-ionized water filtrated through a 0.45 μm filter. The concentration of Gd in each sample was determined using an ICP-AES at 335.047 nm.

2.9. Statistical analysis

The student *t*-test assuming equal variances was used if two groups were to be compared. If more than two groups were involved, the one way analysis of variance (ANOVA) followed by pair-wise comparisons with Fisher's protected least significant difference procedure (PLSD) was used. A p value of < 0.05 (two-tail) was considered to be statistically significant.

3. Results and discussions

The success of the NCT is dependent on the pre-deposition of ^{157}Gd in tumor tissues. The optimal ^{157}Gd concentration in tumors was predicted to be in the range of 50 to 200 μg/g of tumors [9,45]. Delivery of Gd into tumors using particulate carriers with size in the nanometer scale has been suggested to be a potentially feasible approach to accumulate the required amount of Gd in tumor tissues. The reconstituted chylomicron emulsion was prepared with commercially available lipids and oil that are, or can be readily metabolized into, naturally occurring and biocompatible molecules [28]. Thus, safety issues will not be a concern in using this emulsion to deliver drugs. Furthermore, the size of these emulsion particles can be readily reduced to around 100 nm by extruding through filters, making them an attractive carrier for Gd compounds. When the surface of this chylomicron emulsion is modified to prevent the binding of serum proteins, the emulsion may be used to target Gd into solid tumors for potential NCT.

3.1. Incorporation of Gd into the chylomicron emulsion

GdAcAc was selected to be incorporated into the chylomicron emulsion because GdAcAc has very limited water solubility (<0.5 μg/mL) and is a relatively small molecule (MW, 454.57). About 34.59% of the mass in the GdAcAc is from Gd. The reconstituted chylomicron emulsion, which was prepared with olive oil, PC, Lyso-PC, CO, and Chol in a ratio of 70:22.7:2.3:3.0:2.0 (w/w), was readily formed by hydration of the lipid thin film, followed by sequential extrusion through 1.0, 0.4, and 0.1 μm filters. To incorporate GdAcAc into the emulsion, GdAcAc was co-dissolved in chloroform. After the evaporation of the chloroform, the thin lipid film formed was hydrated in de-ionized water, incubated at 65 °C for 5 min, and sonicated in a waterbath sonicator for

about 5 min. An opaque and fluid emulsion was formed for preparations with 5, 10, 15, and 20% of GdAcAc.

3.2. Determination of the incorporation efficiency

Gel permeation chromatography was used to separate GdAcAc-incorporated emulsion particles from unincorporated GdAcAc. By passing Gd-free emulsion particles through the column and measuring the turbidities (UV–vis absorbance at 655 nm) of each fraction eluted out, we conclude that the emulsion particles were mainly eluted out in fraction three (data not shown). Fig. 1 shows the GPC profile of the GdAcAc-incorporated emulsion prepared with 20% (w/w) of GdAcAc. Apparently, the sephadex G-75 column can be used to separate GdAcAc-incorporated emulsion particles from unincorporated GdAcAc, which may include free GdAcAc and GdAcAc solubilized by micelles formed by lipids used in the emulsion preparation. Thus, the GPC was a feasible method to purify the emulsion particles. Based on the GPC graph, the incorporation efficiency of GdAcAc in the emulsion prepared with 20% (w/w) GdAcAc was estimated to be 36.7 ± 2.9%. The incorporation efficiencies for GdAcAc-emulsions prepared with 5%, 10%, and 15% of GdAcAc were determined similarly and are shown in Fig. 2. As predicted, the incorporation efficiency of GdAcAc in the emulsion decreased as the percent of GdAcAc added increased. However, the exact amount of GdAcAc incorporated into the emulsion increased as the percent of GdAcAc initially added increased. Thus, the chylomicron emulsion prepared with 20% (w/w) of GdAcAc (vs. total lipids) had the highest amount of GdAcAc incorporated, which was estimated to be 2.94 ± 0.23 mg/mL, corresponding to about 1.02 mg/mL of pure gadolinium (Gd). In a mouse having

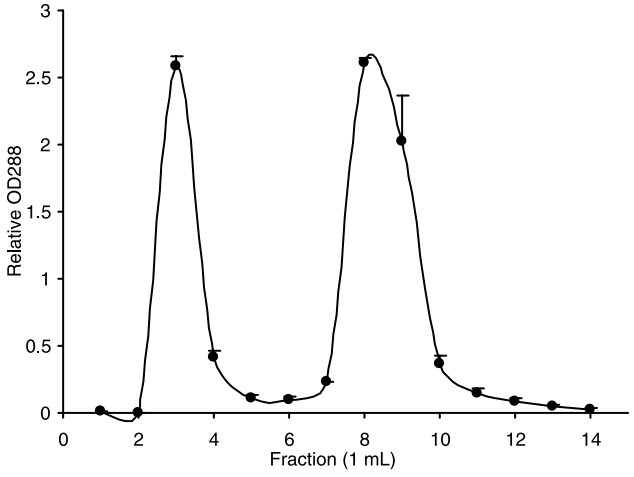

Fig. 1. The unincorporated GdAcAc can be separated from GdAcAc-incorporated emulsion particles by gel permeation chromatography (GPC). The reconstituted chylomicron emulsion (0.1 mL) prepared with 20% of GdAcAc was applied into a sephadex G75 column (diameter = 0.7 cm, length = 25 cm), and eluted with filtrated (0.2 μm) and de-ionized water. The absorption at 288 nm by each elution fraction (1 mL) was measured using a UV-spectrophotometer. The relative OD 288 values are those OD 288 values after being subtracted by the OD 288 values of the corresponding elution fraction of the GdAcAc-free emulsion. Reported are mean ± standard deviation ($n = 3$).

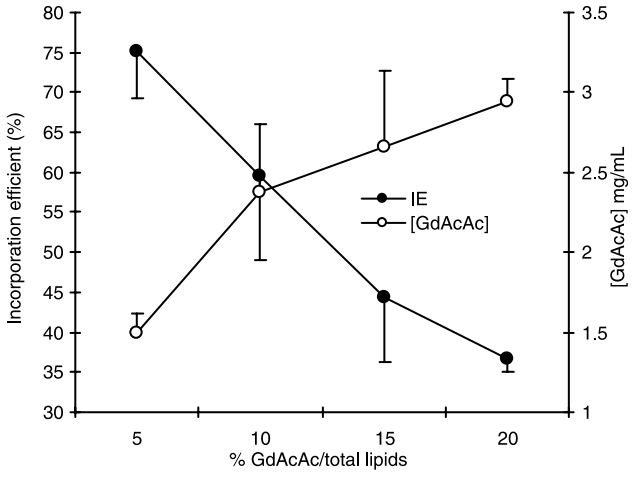

Fig. 2. The incorporation efficiency (IE, ○) and the exact concentration (●) of GdAcAc incorporated inside the chylomicron emulsion varied as the percent of GdAcAc added increased. Chylomicron emulsions were prepared with 40 mg/mL of total lipids and an increasing amount of GdAcAc (5–20%, w/w). The percent of GdAcAc that was incorporated inside the emulsion was calculated and reported as the incorporation efficency. The exact final concentration of GdAcAc that was incorporated in the emulsion was also calculated and reported. All values shown are mean ± standard deviation ($n = 3$).

a 500 mg solid tumor, one single injection of this GdAcAc-emulsion (250 μL) may lead to a sufficient amount of pure Gd in the tumor if around 10% of the injected is accumulated in the tumor. Earlier studies have reported as high as 15% uptake of injected nanoparticles by tumors (Oyewumi et al., 2004). If needed, the concentration of pure Gd may also be increased by ways such as choosing or synthesizing alternative and more lipophilic Gd-containing compounds (e.g. Gd-2,4-hexanedione) [46]. The apparent solubility of the GdAcAc was enhanced by more than 5880-fold, from less than 0.5 μg/mL to close to 3 mg/mL. Because the emulsion prepared with 20% of GdAcAc had the highest amount of GdAcAc incorporated, it was used in all following studies.

The coating of PEG on the surface of the GdAcAc-emulsion was completed by including 2.5% (m/m) of DSPE-PEG (2000) maleimide into the total lipids prior to the emulsion preparation. It was likely that close to 100% of the DSPE-PEG (2000) maleimide added was incorporated into the emulsion. Also, we believe that the PEG was attached on the surface of the emulsion particles via the DSPE moiety, which was inserted into the emulsion due to its lipophilic nature.

3.3. Release of GdAcAc from the emulsion

In order to accumulate a sufficient amount of Gd inside tumors, the release of the GdAcAc from the emulsion should be slow and limited. A slow and limited release will prevent any significant leakage of GdAcAc from the emulsion before it reaches tumors and will allow the GdAcAc that reaches the tumor to remain inside the tumor. As shown in Fig. 3A, the release of GdAcAc from the GPC-purified, PEG-free, GdAcAc-incorporated emulsion was very limited and slow. Only about 5% was released in ~20 h (Fig. 3A). A burst

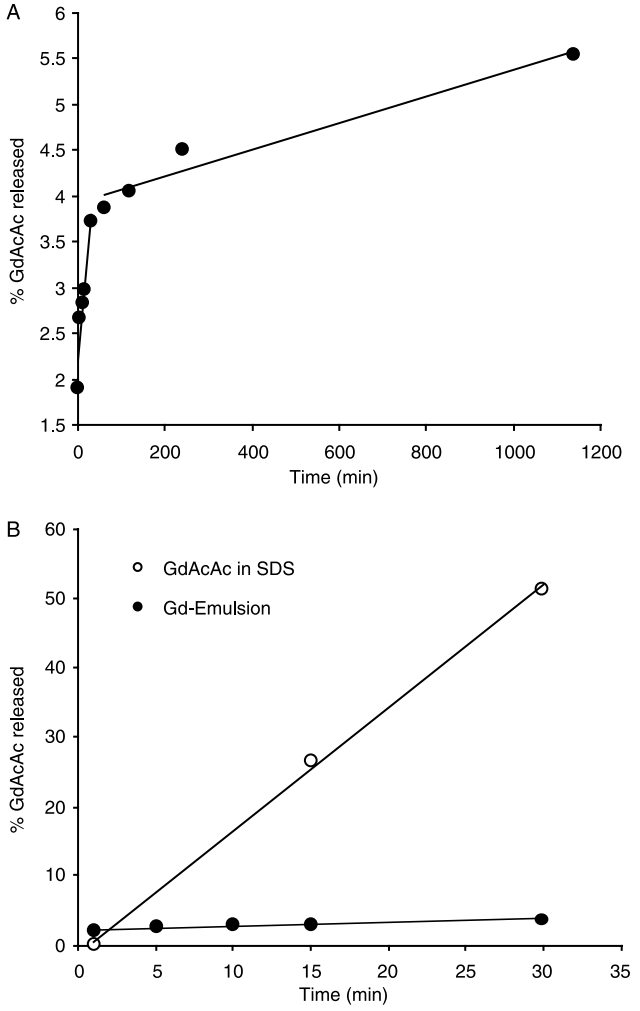

Fig. 3. (A). The release profile of GdAcAc from the chylomicron emulsion. The GPC-purified, GdAcAc-incorporated emulsion was placed into a cellulose ester dialysis tube (molecular weight cut, 3.5 kDa). The dialysis tube was then placed into 50 mL of PBS (pH 7.4, 10 mM) containing 0.5% of SDS as the release medium. The percent of GdAcAc released into the release medium was determined as a function of time. (B) The diffusion of GdAcAc, when dissolved into 0.5% SDS in PBS, from the dialysis membrane (open circles). Shown in filled circles is the observed release of GdAcAc from the emulsion particles. Shown are the mean values from three independent experiments.

release was observed in the initial 30 to 60 min (Fig. 3A). The release was then slowed down. The profile for the release of GdAcAc from the PEG-coated, GdAcAc-emulsion was similar to that for the release of GdAcAc from the PEG-free, GdAcAc-emulsion (data not shown). Although more research needs to be completed to depict the microscopic structure of the emulsion particles, we propose the emulsion as a sphere whose interior is not homogenous. The 'core' should be filled mainly with olive oil, while the 'shell' of the sphere is made of lipids such as PC, lyso-PC, and Chol. GdAcAc could be distributed in the 'core' and the 'shell'. The initial burst release may represent the release of those GdAcAc molecules in the particle 'shell' or loosely associated on the surface. The followed release with a much smaller rate might be the release of those GdAcAc incorporated deeply inside the 'core' of the emulsion particle. Fig. 3B was shown to verify that it was feasible to use the dialysis membrane, in which the GPC-purified, GdAcAc-incorporated emulsion particles were placed, to study the release of GdAcAc from the emulsion because the rate ($\sim$4.28 μg/mL) for GdAcAc to diffuse out the dialysis membrane was much greater than the rate ($\sim 0.51 \pm 0.21$ μg/mL) for the release of GdAcAc from the emulsion in the first 30 min burst release period. This difference in the release rated also pointed out that the burst release observed was unlikely to be from free GdAcAc in the emulsion preparation that was not removed during the GPC purification step.

3.4. Stability of the GdAcAc-incorporated emulsion

The sizes of the GdAcAc-incorporated emulsions, PEG-free or PEG-coated, are listed Table 1. Their size may be further reduced if they are extruded through a filter with a smaller pore size, such as 0.08 μm. The relatively larger size observed for the PEG-coated, GdAcAc-emulsion was likely due to the long polyethylene glycol chains [$(CH_2CH_2O)_{45}$] projecting out on the surface of the emulsion particles. When stored at 4 °C, the size of the emulsion particles did not change significantly in a two-week short-term stability study (data not shown). In Table 1, we observed that the mean size of the PEG-free, GdAcAc-incorporated emulsion doubled when they were incubated in 10% FBS in PBS for 30 min (from 115 to 233 nm), indicating that a small extent of aggregation happened in the incubation period. It takes about eight emulsion particles to aggregate together to double the diameter measured. As predicted, coating of the emulsion with PEG molecules prevented the aggregation because the size of the PEG-coated, GdAcAc-incorporated emulsion did not change after incubation in the same condition. However, it is worth to point out that the polydispersity of the PEG-coated, GdAcAc-incorporated emulsion particles were increased when incubated in PBS with or without FBS, of which more work still needs to be completed to explain. Taken together, we predict

Table 1
The particle size of the emulsions and their stability in simulated biological media

Medium	Particles	0 min	30 min
H_2O	Gd-Emulsion	115.0±36.0 (0.081)	N/D
	PEG-Gd-Emulsion	126.9±28.7 (0.032)	N/D
10% FBS in PBS (v/v)	Gd-Emulsion	186.6±88.0 (1.012)	233.2±110.6 (1.112)
	PEG-Gd-Emulsion	126.9±28.7 (0.032)	111.3±54.3 (1.654)
PBS (10 mM, pH 7.4)	Gd-Emulsion	128.6±51.9 (0.233)	129.9±51.4 (0.208)
	PEG-Gd-Emulsion	128.4±12.9 (0.005)	152.1±74.2 (0.539)

Reported are means (nm)±standard deviations (polydispersity index, PI). One representative of two or three different measurements that led to similar results is shown. Gd-Emulsion is the GdAcAc-incorporated emulsion; PEG-Gd-Emulsion is the PEG-coated, GdAcAc-incorporated emulsion. N/D=not determined.

that the PEG-coated particles will not extensively aggregate or bind to serum proteins when injected into the blood circulation.

3.5. Biodistribution of the GdAcAc-incorporated emulsion

As predicted, a large percent of the PEG-free, GdAcAc-incorporated chylomicron emulsion was accumulated in mouse livers 5 h after i.v. injection (Fig. 4A). Only a very small percentage of it was still in the blood circulation. However, the distribution of the PEG-coated, GdAcAc-emulsion was very different. Only about 20% of it was recovered in the liver, while a significantly higher percent was still circulating in the blood (71.6 ± 18.4%) (Fig. 4A). Fig. 4B clearly shows that the PEG-coated, GdAcAc-emulsion remained circulating in the blood, while the PEG-free, GdAcAc-emulsion particles were mainly accumulated inside the reticuloendothelial systems (RES, mainly liver and spleen). In future studies, the uptake of PEG-coated, Gd-incorporated emulsion particles by solid tumors established in mice will be investigated. We also plan to conjugate tumor-specific ligand(s) on the surface of the emulsion particles to target them to specific tumor cells.

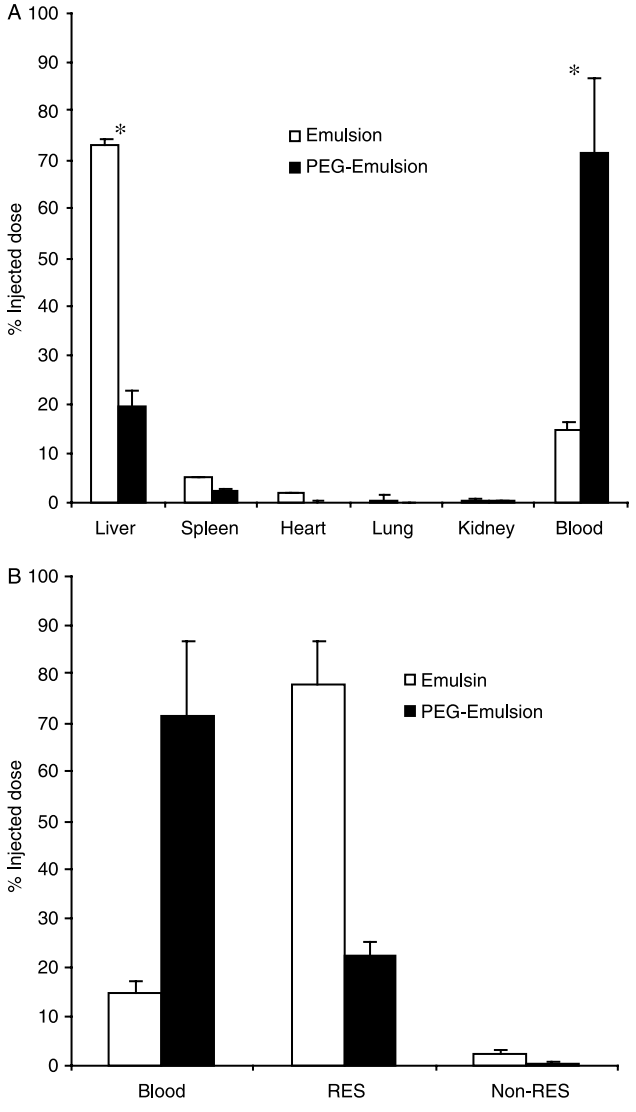

Fig. 4. The biodistribution of GdAcAc in mice. Balb/C mice (n = 4–5) were injected via the tail vein with GPC-purified, GdAcAc-incorporated emulsions (Emulsion, white bars), and GPC-purified, PEG-coated, GdAcAc-incorporated emulsions (PEG-Emulsion, black bars). Five hours after the injection, mice were sacrificed and their liver, spleen, heart, lung, kidney, and blood were harvested. Gd concentration was determined using ICP-AES. (A) Percent of injected Gd recovered from different organs. * indicates the values from the Emulsion were significantly different from that from the PEG-Emulsion. (B) The distribution of injected Gd in blood, RES, and non-RES systems. Spleen and liver were considered to be the RES, and heart, lung, and kidney were considered to be the non-RES. The values from the Emulsion were significantly different from that of the PEG-Emulsions in blood, RES, and non-RES samples.

In summary, we have reported a promising approach to carry Gd nuclide for potential NCT for cancers. This Gd-incorporated reconstituted chylomicron emulsion system has many advantages: (i) the formation of the chylomicron emulsion is a spontaneous assembling process. Thus, any high torque and energy consuming mechanical homogenization procedure required for other emulsion preparations is not required; (ii) the final emulsion is stable, or can be stabilized, when stored in buffers and serum; (iii) all the components in the emulsion are, or can be readily metabolized into, naturally occurring and biocompatible lipids; (v) the Gd-incorporated emulsion may be modified on its surface to obtain a desired biodistribution profile or to target certain cells.

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