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Research paper

Tumor accumulation of gadolinium in lipid-nanoparticles intravenously injected for neutron-capture therapy of cancer

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Abstract

Gadolinium-incorporating lipid-nanoemulsions (Gd-nanoLE) for neutron-capture therapy were prepared. As a more convenient administration route than the intraperitoneal (i.p.) injection previously reported, the intravenous (i.v.) injections in tumor-bearing hamsters were carried out at an administration volume of 1 ml, which was the maximum tolerable injection volume of an i.v. injection and half that of an i.p. injection. When the standard-Gd-nanoLE of 1.5 mg Gd/ml was administered, the absolute bioavailability in the i.p. injection was 57%, probably resulting from incomplete absorption from the peritoneal cavity into the blood stream. The biodistribution data revealed that the i.v. injection had three advantages over the i.p. injection, namely, a faster and higher accumulation of Gd-nano LE, and a more extended retention time in the tumor. Two i.v. injections of the standard-Gd-nanoLE with a 24 h interval doubled the tumor accumulation of Gd, resulting in 49.7 μg Gd/g wet tumor 12 h after administration. By using a twofold Gd-enriched formulation (High-Gd-nanoLE) of 3.0 mg Gd/ml in the repeated administration schedule, the accumulation was doubled again, reaching 101 μg Gd/g wet tumor. This level was comparable to the maximum level in the single i.p. injection previously reported. These results demonstrated that i.v. injection could be an alternative to i.p. injection as an administration route.

Keywords: Gadolinium; Neutron-capture therapy; Cancer therapy; Emulsion; Intravenous injection; Tumor accumulation

1. Introduction

Gadolinium neutron-capture therapy (GdNCT) is a cancer therapy, which uses radiation emitted from gadolinium-157 (^{157}Gd) as a result of its neutron-capture reaction with thermal neutrons. This therapy has certain advantages over traditional cancer chemotherapy. Unlike chemotherapy that uses antitumor drugs, NCT does not need to use pharmacologically active substances in a traditional sense, since it is the neutron-capture element itself that contributes to tumor inactivation. Therefore, the administration of a large amount of the radiosensitizer becomes possible, provided that the elements are modified so as to be non-toxic compounds. Thus, severe side effects, which are often experienced in cancer chemotherapy, are not a major concern in NCT.

The major obstacle to overcome in GdNCT is how to deliver and retain ^{157}Gd, which is the key element of GdNCT, into tumors. In the preliminary studies of GdNCT, the highly hydrophilic gadolinium-diethylenetriaminepentaacetic acid (Gd-DTPA) has been used as a Gd source [1]. However, it is known that intravenously (i.v.) injected Gd-DTPA is rapidly excreted from the systemic circulation, and even intratumorally (i.t.) injected Gd-DTPA cannot be retained at the level that Gd is required for therapeutic effect. In addition, no Gd compound capable of high accumulation in tumors has been developed. Thus, an in vivo Gd-NCT trial was performed in which newly developed Gd-DTPA-loaded chitosan nanoparticles were i.t.-injected into SCC-VII tumor-bearing mice [2]. Although this trial showed that the ^{157}Gd concentration in the tumor required to obtain significant tumor growth suppression was of such a high level as to be more than 100 μg Gd/g wet tumor [2], complete treatment of the cancer was not possible in this trial, probably due to the uneven distribution of Gd in the tumor tissue. These perspectives motivated us to develop a functional Gd device to deliver a sufficient Gd concentration into tumors through the systemic circulation.

In our previous study, Gd-containing lipid-nanoemulsion (Gd-nanoLE), consisting of soybean oil, hydrogenated phosphatidylcholine from egg yolk (HEPC), gadolinium-diethylenetriaminepentaacetic acid-distearylamide (Gd-DTPA-SA) and a co-surfactant with polyoxyethylene

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(POE) unit as a hydrophilic moiety, was designed and prepared to deliver a significant amount of Gd to the tumor [3]. The size of the lipid particles in the Gd-nanoLE was reduced below 100 nm in order to prolong the blood circulation time of Gd and to allow the Gd-nanoLE to easily penetrate the tumor tissues through the discontinuous capillary endothelium in the tumor. In addition, the surface of the lipid particles in the Gd-nanoLE was modified with hydrophilic POE units by using co-surfactants to avoid the rapid removal of the Gd-nanoLE from the circulation by the reticuloendothelial system (RES).

The administration route is one of the most important factors in the biodistribution and pharmacokinetics of drug carriers such as liposomes and lipid-emulsions. In our previous study, the intraperitoneal (i.p.) route was adopted as an administration route of the Gd-nanoLE because it allows the injection of a relatively large amount of the Gd-nanoLE and consequently delivers a large amount of Gd to the tumor via the systemic circulation [4]; as a result, the Gd concentration in the tumor reached 107 μg Gd/g wet tumor at a dose of 6 mg Gd (2 ml as an administration volume of the Gd-nanoLE). However, even with i.p. injection, many factors affect the biodistribution and pharmacokinetics of the drug carriers, including the absorption of these carriers from the abdominal cavity and the localization of these carriers in the lymph nodes, making the estimation of the in vivo fate of the drug carriers difficult. In contrast, i.v. injection delivers drugs more simply and, therefore, has been widely employed instead of i.p. injection in clinical treatments. For these reasons, it was desirable to establish an effective and convenient technique for the administration of the Gd-nanoLE in in vivo GdNCT trials.

The aim of our present study was to evaluate i.v. injection as an alternative to i.p. injection with respect to tumor accumulation of Gd incorporated in Gd-nanoLE. First, the biodistribution of the Gd-nanoLE after i.v. injection was compared with the biodistribution after i.p. injection at the same dose, and, second, the effects of the dosing schedule and the Gd content of the Gd-nanoLE on the biodistribution of Gd after i.v. injection were investigated.

2. Materials and methods

2.1. Materials

Unless otherwise specified, reagents were used as purchased without any purification. HEPC and soybean oil were purchased from Nacalai Tesque Inc., Japan. POE-60 hydrogenated castor oil (Cremophor® RH 60, hereafter abbreviated as HCO-60) (POE MW: 2600, HLB: 14.0) was supplied by BASF Aktiengesellschaft, Ludwigshafen, Germany. Gd-DTPA-SA, which combined highly hydrophilic Gd-DTPA with two hydrophobic stearyl groups through amide linkages, was synthesized as described in our previous report [5].

2.2. Preparation of Gd-nanoLE

The Gd-nanoLE was prepared by a thin-layer hydration method combined with a sonication method. The details of this preparation were described in our previous report [3]. Briefly, soybean oil, HEPC, Gd-DTPA-SA and HCO-60 were dissolved in an appropriate amount of chloroform. To form thin-films, the solvent was removed by a rotary evaporator and dried in a vacuum for 3–4 h at room temperature. The dried films were allowed to hydrate in distilled water at 55–60°C for 5 min. By sonicating the resultant hydrated mixture with a bath-type sonicator and vortexing for 60 min, the Gd-nanoLE was obtained.

2.3. Particle size analysis

The size of the lipid particles in the Gd-nanoLE was measured in distilled water using a dynamic light scattering technique (ZetaPlus with the BI-MAS option, Brookhaven Instruments Co., USA) at 25°C. The lipid particles in the Gd-nanoLE added to the distilled water were well dispersed before the measurements.

2.4. Tissue distribution experiments

Female Syrian (golden) hamsters, 5–6 weeks old, were purchased from Japan SLC, Inc., Shizuoka, Japan. To obtain hamsters bearing Green's melanoma (Melanotic No. 179 cell, D_1-179), which is considered to be a human melanoma counterpart biologically and pathologically, subcutaneous inoculation of the tumor cell fragment ($2 \times 2 \times 2$ mm) into the left nates of the hamsters was performed. When the D_1-179 melanoma tumor in the hamsters grew to about 10 mm in diameter (usually around 10 days after implantation), and the body weight was 90–100 g, the Gd-nanoLE was i.v. injected via the femoral vein of the hamsters or i.p. injected under anesthesia with diethylether. The administration volume of the Gd-nanoLE was fixed at 1 ml per injection (1.5 or 3.0 mg Gd per injection for the standard- or high-Gd-nanoLE, respectively), which was the maximum tolerated volume (MTV) of i.v.-injected Gd-nanoLE per hamster as determined in the preliminary experiment with the hamsters. At a predetermined time after administration, a blood sample was collected by cardiac puncture. The hamsters were then sacrificed with diethylether, and tissue samples including the liver, spleen, kidney, lung and tumor were removed immediately. The skin and muscle were removed from the left nates of the hamsters. At the same time, these tissues were directly wet-ashed with nitric acid, and then the Gd concentrations in each tissue were determined by inductively coupled plasma-atomic emission spectrometry (ICP-AES, P-5200 ICP System, Hitachi Co., Ltd., Tokyo, Japan) at 355.047 nm.

2.5. Pharmacokinetic analysis

Using the mean value of plasma concentration at each

time point, the area under the plasma concentration–time curve (AUC) and the mean residence time (MRT) were determined by the trapezoidal method up to infinite time using the terminal slope on a logarithmic scale.

3. Results

3.1. Preparation of Gd-nanoLE

Table 1 shows the formulation, Gd content and particle size of the standard- and high-Gd-nanoLE. HEPC and soybean oil were used as the emulsifier and oily core component of the Gd-nanoLE, respectively. Gd-DTPA-SA, which was designed to be incorporated in an interfacial layer of lipid particles in the Gd-nanoLE, was used as a Gd source. In order to reduce the particle size and modify the surface of the lipid particles in the Gd-nanoLE, HCO-60 with hydrophilic POE units in each molecule was used as a co-surfactant [3,4]. High-Gd-nanoLE was prepared by changing the weight ratio of HEPC to Gd-DTPA-SA from that of the standard-Gd formulation. Theoretically, the Gd-nanoLE prepared in the standard- and high-Gd formulations contained Gd at 1.5 and 3.0 mg Gd/ml, respectively.

The sizes of the lipid particles in the Gd-nanoLEs are also listed in Table 1. Standard-Gd-nanoLE and high-Gd-nanoLE having particle sizes smaller than 100 nm were successfully obtained.

3.2. Comparison of i.v. with i.p. injection of Gd-nanoLE

The biodistribution of Gd after i.v. or i.p. injection of the standard-Gd-nanoLE with HCO-60 (particle size, 73 nm) at a dose of 1.5 mg Gd is shown in Fig. 1. The calculated pharmacokinetic parameters are summarized in Table 2. The Gd concentration in the blood after i.v. injection of the Gd-nanoLE consistently decreased from the initial high concentration of 136 μg Gd/ml blood at the first sampling time, whereas i.p. injection showed a peak concentration of 50 μg Gd/ml blood at 4 h after administration (Table 2). The bioavailability of Gd after i.p. injection, as calculated from the AUCs, was 57% of that of the i.v.-injected Gd-nanoLE (Table 2).

Table 1
Formulation, Gd content and particle size of the standard- and high-Gd-nanoLE

	Formulation	
	Standard-Gd-nanoLE	High-Gd-nanoLE
HEPC[a] (mg)	500	250
Gd-DTPA-SA (mg)	250	500
Soybean oil (ml)	2	2
Co-surfactant (mg)	750	750
Water (ml)	23	23
Gd-content[b] (mg/ml)	1.5	3.0
Particle size[c] (nm)	73.4 ± 5.3	90.1 ± 5.1

[a] L-Phosphatidylcholine hydrogenated from egg yolk.
[b] Theoretical Gd content.
[c] Each value represents the mean ± SD ($n = 3$).

In terms of the tumor accumulation of Gd, i.v. injection of the Gd-nanoLE had three advantages over i.p. injection, namely, faster accumulation, higher Gd concentration and a more extended retention time of Gd (Fig. 1). The Gd concentration in the tumor after i.v. injection rapidly increased for 6 h after administration, and thereafter it increased further, though very gradually, over the next 24 h. In contrast, the Gd concentration after i.p. injection remained at a low level for the first 6 h, peaked at 12 h and subsequently decreased. The maximum Gd tumor concentrations after i.v. and i.p. injection of the Gd-nanoLE were 29.7 μg Gd/g wet tumor at 24 h and 21.6 μg Gd/g wet tumor at 12 h, respectively.

The i.v. injection of the Gd-nanoLE led to a higher Gd uptake in the liver and spleen (Fig. 1). The Gd concentrations in both of these tissues after i.v. injection always showed higher values when compared to the concentrations after i.p. injection. In the kidney and lung, a higher Gd concentration after i.v. injection was observed for the first 6 h. In the muscle, the Gd concentration of the Gd-nanoLE after i.v. injection was lower than after i.p. injection, whereas that in the skin was comparable.

3.3. Effect of dosing frequency and Gd content of Gd-nanoLE on Gd accumulation in tissues

In order to achieve a higher Gd concentration in the tumor, two i.v. injections at a 24 h interval were carried out by using the standard- and high-Gd-nanoLE. Biodistribution was determined at 12 h after administration (Table 3), because the tumor accumulation almost leveled thereafter with Gd-nanoLEs prepared with HCO-60 (Fig. 1) [4]. Two i.v. injections of the standard-Gd-nanoLE made the Gd concentration in the tumor higher, reaching a level of 50 μg Gd/g wet tumor at 12 h after the second injection. Unfortunately, Gd concentrations in the liver and spleen concurrently increased to almost twice those observed after a single i.v. injection.

Next, two i.v. injections of the high-Gd-nanoLE were carried out in the same dosing schedule at a dose of 3.0 mg Gd per each injection (Table 3). By using this formulation, the Gd concentration in the tumor became almost twice as high as that of the standard-Gd-nanoLE. At 12 h after the last administration, the Gd concentration in the tumor reached 101 μg Gd/g wet tumor.

4. Discussion

In our previous studies, the biodistribution of the Gd-nanoLE after i.p. injection at a dose of 6.0 mg Gd (2 ml as an administration volume of the Gd-nanoLE) per hamster was investigated in tumor-bearing hamsters [4]. Through these studies, the utility of the Gd-nanoLE was supported,

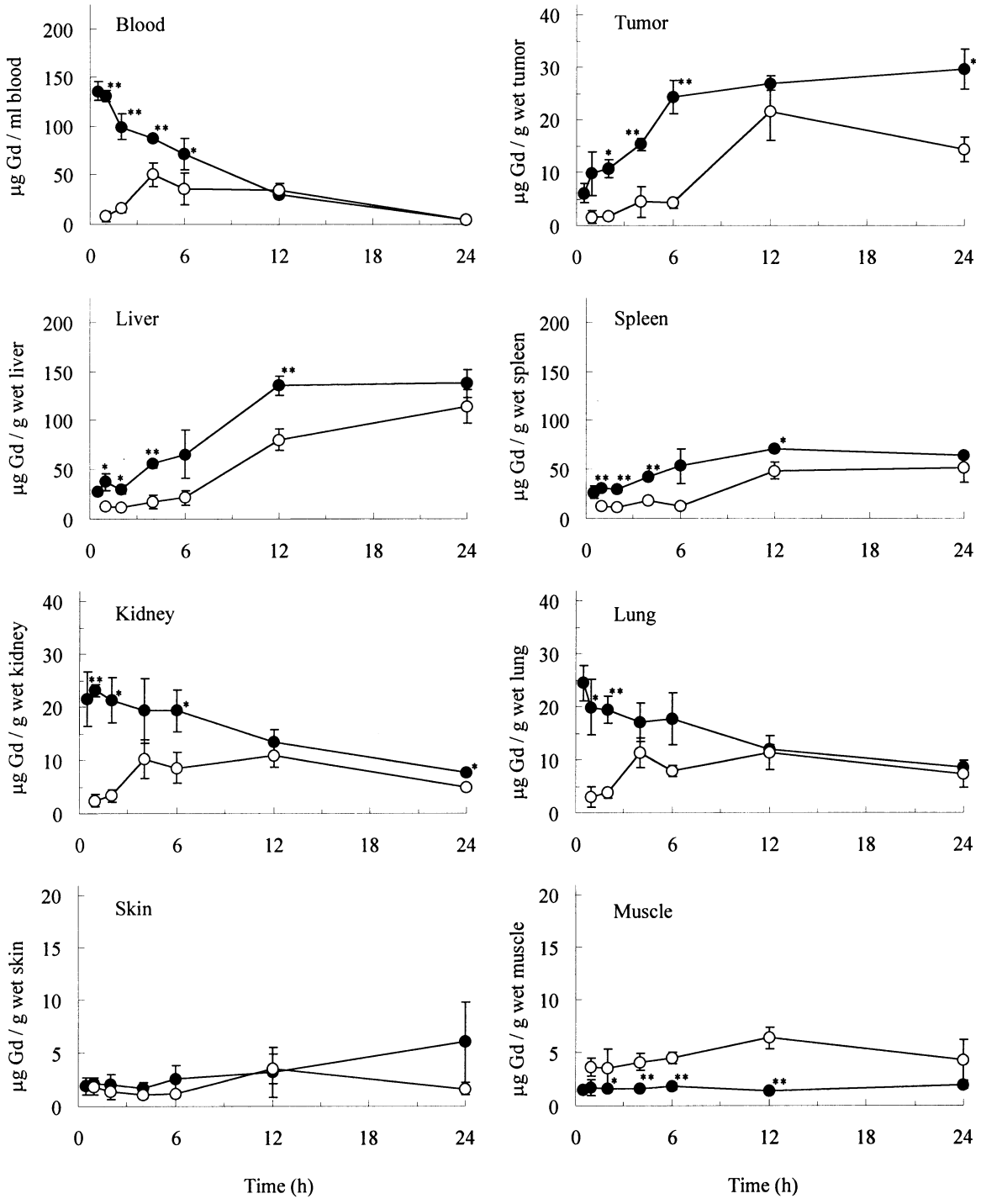

Fig. 1. Tissue distribution of Gd after i.p. or i.v. administration of the standard-Gd-nanoLE. (open circle) i.p. administration; (closed circle) i.v. administration. Dose: 1.5 mg Gd/hamster. Each value represents the mean ± SD ($n = 3$–5). *$P < 0.05$ and **$P < 0.01$, significantly different from the Gd concentration of the i.p. injection.

at least with regard to the accumulation of Gd in the tumor. In the present study, the biodistribution of the Gd-nanoLE after i.v. injection was investigated to find an alternative route of administration that may be more convenient for in vivo GdNCT trials.

In the comparative study between i.v. and i.p. injection of the Gd-nanoLE, Gd concentration–time profiles in the blood were found to differ according to the injection route (Fig. 1). The AUC of Gd after i.p. injection was only 57% of the AUC of Gd after i.v. injection (Table 2). It was earlier reported that the blood concentration of a marker incorporated into the liposomes containing sphingomyelin and

Table 2
Pharmacokinetic parameters after single i.p. or i.v. administration of the standard-Gd-nanoLE with HCO-60 at a dose of 1.5 mg Gd

Route	C_{max}[a] (μg/ml)	T_{max}[a] (h)	AUC[b] (μg h/ml)	MRT[b] (h)
i.p.	50.0	4.0	612	9.2
i.v.	(135.9)	(0.5)	1071	6.3

[a] The maximum blood concentration, C_{max}, and the time of the maximum blood concentration, T_{max}, were derived directly from the mean blood concentration–time curve.
[b] Each value was calculated from the mean blood concentration values ($n = 3$–5).
The values in parentheses were obtained from the corresponding data point shown in Fig. 1.

cholesterol after i.p. injection peaked at several hours after administration and subsequently declined [6]. In addition, some reports have shown that the absorption route of the conventional liposomes from the peritoneal cavity to the bloodstream is mediated by the lymphatic system [7,8], and a part of the i.p. injected liposomes are localized in the lymphatic system over 24 h [8]. These findings can probably be extended to interpret the behavior of the lipid-nanoemulsions in the peritoneal cavity. Accordingly, the delayed increase of Gd concentration in the blood after i.p. injection might be ascribed to the transport time of the Gd-nanoLE from the peritoneal cavity to the bloodstream through the lymphatic system. In addition, the lower AUC of Gd after i.p. injection, compared with i.v. injection, might be explained by the localization of a partial Gd-nanoLE in the lymphatic system, since the Gd-nanoLE remaining in the peritoneal cavity, as determined by visual observation, was negligible at 12 h after administration.

The i.v. injection of the Gd-nanoLE always resulted in a higher Gd concentration in the tumor over 24 h than did the i.p. injection (Fig. 1). At 24 h after administration, the Gd concentration level in the tumor after i.v. injection was almost twice as high as that after i.p. injection. Further, the extended retention time and the high concentration of the radiosensitizer in the tumor are desired in NCT, because these conditions allow repeated irradiation of the thermal neutron to the tumor containing the radiosensitizer, giving rise to a more valid treatment efficacy. Again, the i.v. injection had an obvious advantage over i.p. injection in terms of the retention of Gd in the tumor. From the results of the distribution in the blood and tumor shown in Fig. 1, it can be concluded that a 1 ml dose corresponding to the MTV in the i.v. injection of the Gd-nanoLE was superior to the i.p. injection for the purpose of efficiently utilizing Gd for an in vivo NCT trial.

Table 3
Effect of dosing frequency and Gd content of the Gd-nanoLE with HCO-60 on the tissue distribution of Gd (μg Gd/wet tissue) at 12 h after the final i.v. administration at a dose of 1.5 mg Gd per injection for the standard- or 3.0 mg Gd per injection for the high-Gd formulation

Tissue	Standard-Gd-nanoLE		High-Gd-nanoLE
	Single (1.5)[a]	Double (3.0)[a]	Double (6.0)[a]
Blood	29.7 ± 2.8	25.3 ± 5.7	45.1 ± 11.4*
Tumor	26.9 ± 1.4	49.7 ± 30.9	100.7 ± 35.9*
Liver	135.8 ± 9.7	275.3 ± 34.1	540.1 ± 84.4**
Spleen	70.7 ± 2.7	205.7 ± 93.5	437.1 ± 47.1*
Kidney	13.4 ± 2.4	26.8 ± 9.5	20.7 ± 2.2
Lung	12.0 ± 0.7	21.4 ± 2.0	38.3 ± 10.5*
Skin	3.2 ± 2.4	11.9 ± 5.0	14.2 ± 7.5
Muscle	1.4 ± 0.2	7.1 ± 3.9	7.8 ± 0.9

[a] The values in parentheses were total dose of Gd (mg). Each value represents the mean ± SD ($n = 3$–5). *$P < 0.05$ and **$P < 0.01$, significantly different from the Gd concentration of the standard-Gd-nanoLE injected twice.

In NCT, thermal neutrons are irradiated from outside the body. Therefore, the tumor/skin ratio is an important factor affecting the therapeutic potency of NCT. In boron NCT, Mishima [9] reported that when boronophenylalanine-fructose complex was administered by the drip infusion method, the average tumor/skin ratio of the boron concentration was estimated to be 3–6. In the present study, the tumor/skin ratio of the Gd concentration was almost comparable to that in Mishima's case (Table 3). This result suggests that skin damage upon thermal neutron irradiation is probably avoidable.

Two i.v. injections of the standard-Gd-nanoLE made the Gd concentration in the tumor higher: 50 μg Gd/g wet tumor at 12 h post-second administration (Table 3). In certain cases of the repeated administration of liposomes, an accelerated blood clearance and altered biodistribution of the liposomes were observed at the second or later administration for a certain period after the administration, resulting from the induction of an immunoreaction [10]. On the other hand, Oussoren and Storm [11] demonstrated that the kinetic profiles of the first and second injections of the PEG-liposomes with a 24 h interval were virtually identical. In our case, the Gd concentrations in tissues after two administrations of the standard-Gd-nanoLE seemed to be almost twice as high as those after a single administration (Table 3). This implied that, by employing the same schedule as Oussoren and Storm, the repeated administration had no significant effect on the biodistribution mechanisms of the Gd-nanoLE.

With the administration of two i.v. injections of the high-Gd-nanoLE, the Gd concentration in the tumor reached 101 μg/g wet tissue (Table 3). Many researchers have demonstrated that the charge and fluidity of the liposomal membrane affected the biodistribution even in conventional liposomes [12,13]. For instance, Nagayasu et al. [13] reported that the tumor-to-bone marrow accumulation ratio of the HEPC-containing liposomes increased remarkably with a decrease in cholesterol content. In the present study, it was anticipated that the membrane property of the high-Gd-nanoLE would differ from that of the standard-Gd-nanoLE. However, the biodistribution of Gd-nanoLE after i.v. injection of each formulation was likely to be almost identical (Table 3), as it had been after i.p. injection [4]. Thus, the biodistribution of the Gd-nanoLE was hardly

affected by the membrane property. In concurrence, it was also observed that the Gd tumor concentration was proportional to the Gd content of the lipid particles in the Gd-nanoLE. According to this finding, a higher Gd tumor concentration could be achieved by loading a highly lipophilic Gd compound into the core component, though the further introduction of the Gd-DTPA-SA into the membrane of the Gd-nanoLE would lead to instability of the emulsion.

The Gd concentration obtained by two i.v. injections of 1 ml of the high-Gd-nanoLE at a dose of 3.0 mg Gd per injection, namely, 101 μg Gd/g wet tissue, was comparable to the level achieved by a single i.p. injection of 2 ml of the high-Gd-nanoLE at a dose of 6.0 mg Gd per injection [4]. These results indicated that even i.v. injection of the Gd-nanoLE can result in the accumulation of Gd in the tumor at a high concentration when an appropriate dosing schedule is employed.

5. Conclusion

In the present study, the biodistribution of the Gd-nanoLEs after i.v. injection was investigated. The Gd concentration–time profiles in the blood were found to differ greatly depending on the administration route. In terms of the tumor accumulation of Gd, i.v. injection of the Gd-nanoLE had three advantages over i.p. injection, namely, faster accumulation, higher Gd concentration and a more extended retention time of Gd. The enrichment of Gd in the Gd-nanoLE led to the increased Gd accumulation in the tumor. After two i.v. injections of the high-Gd-nanoLE were administered, the Gd tumor concentration reached 101 μg Gd/g wet tissue. These results indicate that i.v. injection could be used as an alternative method of administering the Gd-nanoLE in GdNCT.

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