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Internal radiotherapy and dosimetric study for $^{111}In/^{177}Lu$-pegylated liposomes conjugates in tumor-bearing mice

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Abstract

In vivo characterization and dosimetric analysis has been performed to evaluate the potential of pegylated liposomes as carriers of radionuclides in tumor internal radiotherapy.

Methods: The DTPA/PEG-liposomes were synthesized with a medium size of 110 nm, conjugated with $^{111}In/^{177}Lu$-$(oxine)_3$ to afford $^{111}In/^{177}Lu$-liposome. The stability of $^{111}In/^{177}Lu$-liposome in serum was investigated. The biodistribution, scintigraphic imaging and pharmacokinetics of $^{111}In/^{177}Lu$-liposomes after intravenous(i.v.) injection into C-26 tumor-bearing BALB/cByJ mice were studied. Radiation dose was estimated by MIRD-III program.

Results: The incorporation efficiency of $^{111}In/^{177}Lu$ into liposomes was 95%. After incubation at 37 °C for 72 h in serum, more than 83% of radioactivity was still retained in the intact $^{111}In/^{177}Lu$-liposomes. The biodistribution of ^{111}In-liposomes showed that the radioactivity in the blood decreased from 23.14 ± 8.16%ID/g at 1 h to 0.02 ± 0.00%ID/g at 72 h post-injection (p.i.), while reaching its maximum accumulation in tumors at 48 h p.i., with half-life in blood of 10.2 h. The results were supported by that of ^{177}Lu-liposomes. Scintigraphic imaging with ^{111}In-liposomes showed unambiguous tumor images at 48 h p.i. Dose estimation showed that the absorbed dose in tumor from ^{177}Lu-liposomes was 5.74×10^{-5} Gy/MBq.

Conclusions: This study provides an in vivo characterization and dosimetric evaluation for the use of liposome systems as carriers in targeted radionuclide therapy. The results suggest that adequate tumor targeting as well as dose delivered to tumors could be achieved by the use of radionuclide targeted liposomes.

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1. Introduction

Liposomes have been widely studied as important carriers in controlling the spatial and temporal distribution of bioactive molecules for targeted therapy [1]. Encapsulation in liposomes protects the drug from degradation and improves its pharmacokinetic profile [2]. Research performed during the past two decades has provided a better understanding of the influence of liposome's characteristics on their circulation kinetics and tissue distribution after i.v. injection [3]. Their application in drug delivery has been made possible by using polyethylene glycol (PEG) chains

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to reduce uptake and catabolism of liposomes by the reticular endothelium system (RES), thereby increasing circulatory half-life. Liposomes may be used to deliver therapeutic agents, including radionuclides [4]. The surface of liposomes can be modified with different functional groups (antibodies, peptides, etc.), enabling radiolabeled liposomes to be used for molecular imaging and targeted radionuclide therapy [1].

^{111}In-labeled molecules are generally used as chemical and biological surrogates to study biodistribution and estimate radiation dosimetry of ^{90}Y-labeled molecules [5]. Compared with ^{90}Y, ^{177}Lu, a lanthanide radiometal, is a low-energy β^--emitter (maximum β^-, 0.497 MeV) with small γ-component (133 keV, 6.5%; 208 keV, 11%) suitable for scintigraphic imaging without using a radionuclide surrogate [6]. In this study, in vivo characterization and dosimetry estimation were performed to evaluate the potential of pegylatedliposomes as carriers of radionuclides in tumor targeted radiotherapy.

2. Materials and methods

2.1. Preparation of DTPA-liposome

Diethylenetriaminepentaacetic acid was entrapped within a pegylated liposome matrix combination into small unilamellar vesicles (SUV, size <100 nm) using the standard thin-film hydration and repeated extrusion method. The liposomal salt in excess was removed by a Sephadex G-50 column eluted with histidine-sucrose buffer.

2.2. Radiolabeling of ^{111}In/^{177}Lu-oxine

A total of 300 μL of 68 mM 8-hydroxyquinoline (oxine; Sigma-Aldrich Co., St. Louis, MO, USA) in ethanol was added to 300 μL of $^{111}InCl_3$ (Perkin Elmer, Boston, MA) in 0.05 M sodium acetate buffer (pH 6–7) and then incubated at 50° for 30 min. The lipophilic components were extracted with methylene chloride; the organic layer was then dried with anhydrous sodium sulfate. The labeling efficiency of ^{111}In-oxine was determined by instant thin layer chromatography (ITLC). ^{177}Lu-oxine was prepared and analyzed following the same procedure. The radiochemical yield was generally greater than 90% for ^{111}In-oxine and about 70% for ^{177}Lu-oxine.

2.3. Preparation of ^{111}In/^{177}Lu-DTPA-liposomes (^{111}In/^{177}Lu-liposomes)

^{111}In-oxine was incubated with liposome for 30 min at 37°. EDTA was added to chelate any residual free ^{111}In. The entrapment of ^{111}In within the DTPA-liposome was assayed by using a Sephadex^{TM} G-50 Fine (Amersham Biosciences, Pittsburgh, PA, USA) column. ^{177}Lu-liposome was prepared and analyzed following the same procedure. The entrapment of both ^{111}In and ^{177}Lu was typically found greater than 90%.

2.4. In vitro stability of ^{111}In/^{177}Lu-liposomes in human serum

A solution of ^{111}In/^{177}Lu-liposome (1.85'MBq) was added to 5 mL of human serum and incubated at 37 °C. Aliquots of 500 μL were sampled at 5 min, 0.5, 24, 48 and 72 h and the radiochemical purity was then assayed by using a Sepharose-4B column.

2.5. Cell culture and CT-26 colorectal tumor-bearing mouse model

Male BALB/cByJ mice (20–25 g) were inoculated with 5×10^5 colorectal cancer CT-26 cells subcutaneously in the right hind limb. The size of tumors was 562 ± 142 mm^3 (size estimation based on: $4/3\pi\cdots r_1r_2r_3$, r is the radius), 12 days after inoculation.

2.6. Biodistribution and pharmacokinetics of ^{111}In/^{177}Lu-liposomes in CT-26 tumor-bearing mice

The animal experiments were approved by the Institutional Animal Care and Use Committee of the National Yang-Ming University. Tumor-bearing mice were i.v. injected with 3.7 MBq of ^{111}In/^{177}Lu-liposomes via tail vein. Three mice were killed at 1, 4, 24, 48 and 72 h p.i. The radioactivity of tumor and normal tissues was normalized as percentage injected dose per gram of tissue (%ID/g).

For the pharmacokinetic study, tumor-bearing mice were i.v. injected with 3.7 MBq of ^{111}In/^{177}Lu-liposomes or unencapsulated ^{111}In-DTPA via a lateral tail vein. Venous blood samples were obtained and assayed for radioactivity at 16 time points over 72 h.

2.7. Planar γ camera imaging

Tumor-bearing mice were i.v. injected with 3.7 MBq of ^{111}In-liposome through tail vein. A γ camera (E. Cam Multiangle Cardiac, Siemens, Munich, Germany) equipped with a 4 mm pinhole collimator was used for the γ imaging. The images were acquired in a 256×256 matrix for 15 min, at 24 and 48 h p.i.

2.8. Radiation dosimetry

The tumor mass was assumed to be 20 g. The absorption doses to tumor and normal organs were estimated following the radiation dose formula ($\bar{D}h=\Sigma\tau_i\times S_i$). The specific τ value on each source organ was derived, based on the biodistribution and mathematical model. The specific S value on each target-source organ pair was obtained from MIRD-III program.

Table 1
Biodistribution of ^{111}In- and ^{177}Lu-liposomes in BALB/cByJ mice (data expressed in %ID/g, mean±SD, $n = 3$)

	%ID/g of ^{111}In-liposomes (mean±SD)					%ID/g of ^{177}Lu-liposomes (mean±SD)				
Tissue	1 h	4 h	24 h	48 h	72 h	1 h	4 h	24 h	48 h	72 h
Blood (B)	23.14±8.16	20.77±2.47	3.61±2.15	1.80±0.34	0.02±0.00	29.06±5.06	22.03±4.55	4.24±1.15	2.32±0.41	0.05±0.03
Heart	1.56±0.48	1.67±0.16	0.90±0.34	0.50±0.25	0.24±0.01	2.07±0.28	1.65±0.39	1.17±0.46	0.79±0.51	0.57±0.31
Lung	4.89±1.54	5.10±1.30	1.93±0.60	0.65±0.08	0.19±0.03	7.55±2.34	4.95±1.37	1.99±0.87	1.25±0.53	0.53±0.03
Liver	4.01±0.83	6.68±0.56	8.23±2.31	9.96±0.90	5.53±1.60	5.63±1.46	7.23±2.33	14.99±4.20	15.63±4.84	6.17±1.59
Spleen	3.92±1.31	6.94±1.29	8.55±2.59	9.20±0.67	6.99±1.35	6.11±1.71	8.84±4.14	17.88±5.23	17.54±6.61	10.39±3.41
Kidney	4.43±1.52	3.42±0.53	4.95±1.28	5.06±1.78	1.55±0.19	5.94±0.74	4.27±2.18	4.98±2.10	4.50±0.81	2.82±1.27
Bone	0.97±0.30	1.11±0.16	0.93±0.44	0.99±0.06	0.57±0.12	1.52±0.45	1.30±0.57	1.63±0.44	1.46±0.25	1.00±0.45
Tumor (T)	0.66±0.22	1.51±0.16	3.95±1.22	4.14±0.71	1.79±0.54	0.61±0.32	1.53±0.60	3.97±1.75	4.68±1.55	3.23±1.70
T/B	0.03	0.07	1.09	2.30	89.50	0.02	0.07	0.94	2.02	64.60

3. Results

The preparation of ^{111}In/^{177}Lu-liposome was conducted with high radiochemical yield (70–80%) and high radiochemical purity (>90%). After incubation in human serum at 37° for 72 h, ^{111}In/^{177}Lu-liposomes accounted for more than 83% radioactivity in the serum and ^{111}In/^{177}Lu-DTPA accounted for the rest. ^{111}In/^{177}Lu-liposomes demonstrated virtually the same pattern of biodistribution and pharmacokinetics (Table 1). The maximum accumulations of the radioactivity in tumors observed at 48 h p.i. indicated a long in vivo circulation of the liposome even at low PEG formulation (PEG was less than 1 mol% of total lipid). The tumor-to-blood ratio increased steadily with time up to 72 h. The half-lives of ^{111}In- and ^{177}Lu-liposomes in blood were 10.2 and 11.5 h, respectively (Fig. 1). By contrast, the radioactivity of ^{111}In-DTPA in blood decreased dramatically after injection.

Although the highest accumulation of ^{111}In-liposomes was in liver and spleen, the tumor still could be clearly visualized in scintigraphic images (Fig. 2) up to 48 h p.i. The permanence time of ^{111}In- and ^{177}Lu- liposomes in tissues was similar (Table 2). The radiation doses of ^{177}Lu-liposomes were about fourfolds higher than that of ^{111}In-liposomes due to the β-particle emission of ^{177}Lu.

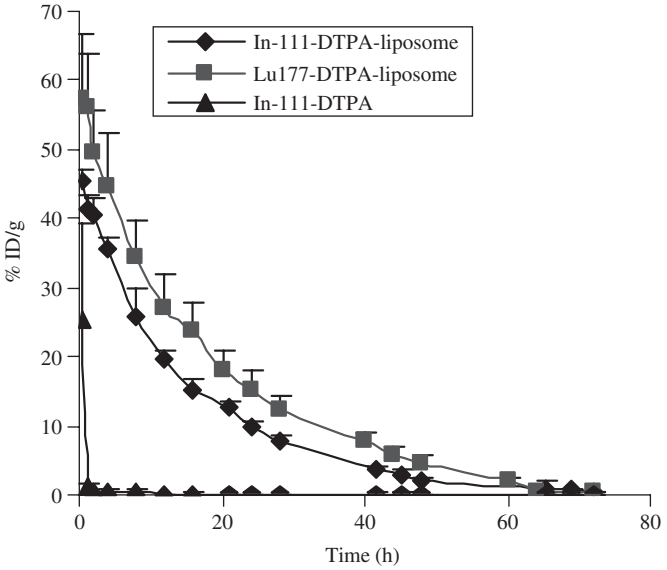

Fig. 1. The radioactivity in the blood of BALB/cByJ mice after i.v. injection of ^{111}In/^{177}Lu-liposomes and unencapsulated ^{111}In-DTPA (data expressed in percentage injection dose per gram, mean±SD, $n = 3$).

4. Discussion

The aim of the present study was to investigate the biodistribution and dosimetry of ^{111}In/^{177}Lu-liposomes in our mouse tumor model to assess their possible role as cancer imaging/therapeutic agents. This study demonstrates prolonged retention of radiolabeled lightly pegylated liposomes within the tumor after i.v. injection and confirms the capability of ^{111}In/^{177}Lu-liposomes to target CT-26 tumors in mice. Biodistributions of ^{111}In/^{177}Lu-DTPA-liposomes differ from those of unencapsulated ^{111}In/^{177}Lu-DTPA, which is rapidly cleared from circulation (Fig. 1) with no evidence of progressive accumulation in either tumor or normal tissues.

The incubation of ^{111}In/^{177}Lu-liposomes in human serum showed only little leakage of radioactivity to serum fractions. Harrington et al. [7] demonstrated the released ^{111}In remained tightly chelated to DTPA with no evidence of significant in vitro or in vivo transchelation of ^{111}In to serum proteins for either ^{111}In-DTPA-labeled pegylated liposomes or free^{111}In-DTPA.

Thus, the measured radioactivity within the tumor and other tissues can reliably be considered to represent liposome-encapsulated radioactivity, and the possibility of the uptake to be due to targeting by radiolabeled serum elements, such as transferrin, can be eliminated. The results derived from this study may provide a useful predictor of possible adverse effects by lightly pegylated liposomal drug formulations, guiding the design and development of novel targeted strategies. Although, these studies provide no information on the microscopic localization of the radioactivity, it is possible that the majority of radioactivity remained within the liposomes in the extracellular space. The results of biodistribution (Table 1) showed that the radioactivity of ^{111}In and ^{177}Lu accumulated in tumor

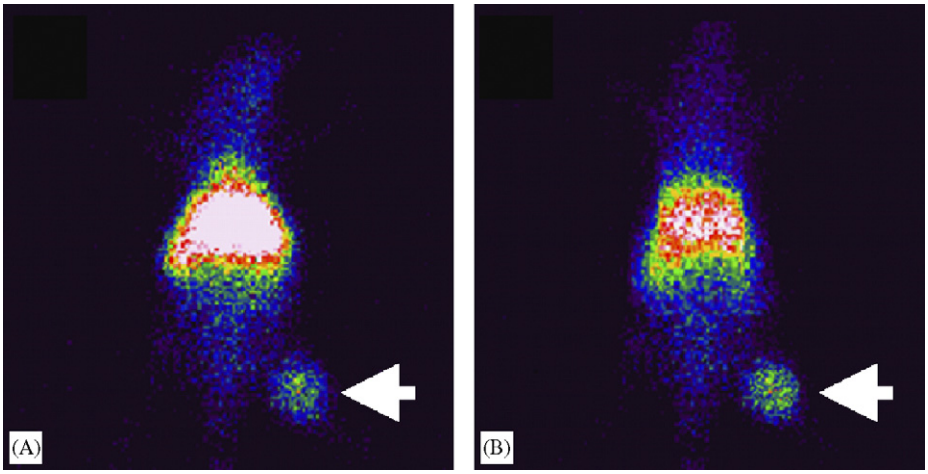

Fig. 2. Whole-body scintigraphy of BALB/cByJ male mice bearing CT-26 carcinoma inoculated subcutaneously at right hind limb (arrow) at 24 h (A) and 48 h (B) after intravenous injection of approximately 3.7 MBq of ^{111}In-liposome. The animals were under isoflurane anesthesia and the image acquisition time was 15 min.

Table 2
Estimated radiation doses (unit: Gy/MBq) to tumor and normal organs after i.v. injection of ^{111}In/^{177}Lu-liposomes

		Tissue/organ					
		Critical organ 1 (Heart wall)	Critical organ 2 (Red marrow)	Liver	Spleen	Kidneys	Tumor
^{111}In-DTPA-liposome	Residence time (h)	0.14	0.45	3.45	0.38	0.29	0.013
	Radiation dose	1.87×10^{-4}	4.30×10^{-6}	3.61×10^{-5}	4.10×10^{-5}	1.95×10^{-5}	1.85×10^{-5}
^{177}Lu-DTPA-liposome	Residence time (h)	0.13	0.34	3.16	0.39	0.23	0.011
	Radiation dose	6.59×10^{-4}	8.75×10^{-5}	1.49×10^{-4}	1.88×10^{-4}	6.97×10^{-5}	5.74×10^{-5}

increased to a maximum at 48 h p.i. (4.14 and 4.68%ID/g, respectively) and this was in agreement with planar γ imaging (Fig. 2).

After intravenous administration, plasma clearance kinetics of ^{111}In- and ^{177}Lu-labeled liposomes from circulation were similar (Fig. 1). The biological half-life of these two radiopharmaceuticals in blood (10.2 and 11.5 h, respectively) showed no statistically significant difference. Biodistribution study (Table 1) also showed that the distribution patterns in tissues were similar between ^{111}In- and ^{177}Lu-labeled liposomes.

The development of radiolabeled drugs as therapeutic agents involves estimation of radiation absorption dose as part of safety assessment in clinical trials. Knowledge of radiation doses to various critical organs is crucial for a better understanding of dose–response relationship between myelotoxicity and second-organ toxicity. Quantitative dosimetric imaging and pharmacokinetic studies of the radiolabeled therapeutic agents in a MIRD schema are essential to accurately determine the relationship between time and radioactivity in organs and to calculate its permanence time in tissues [5]. For both ^{111}In- and ^{177}Lu-labeled liposomes, heart wall is the critical target tissue, followed by spleen, liver, kidneys and tumor (Table 2). The dose to heart wall from ^{177}Lu- (0.66 mGy/MBq) was 3.5-folds higher compared with that from ^{111}In-derivatives (0.19 mGy/MBq). Similarly, for other source organs (liver, spleen and kidneys), the doses from ^{177}Lu were about fourfolds higher when compared with those from ^{111}In-derivatives. A higher radiation dose to source organs with ^{177}Lu is understandable, since the equilibrium dose constant (rad g/h) for β^--particles is 0.284 for ^{177}Lu compared to 0.117 for ^{111}In. In contrast, the equilibrium dose constant for ^{111}In-species γ-photons (0.822) is about 11 times greater when compared with that for ^{177}Lu-derivatives (0.075). The doses to liver, spleen, kidneys and tumor are comparative suggesting that ^{111}In/^{177}Lu-labeled liposomes used in this study might serve as means of delivering a local radiation boost to the residual tumor and metastatic lymph nodes after external beam radiotherapy.

5. Conclusion

^{111}In- and ^{177}Lu-labeled pegylated liposomes have similar plasma clearance kinetics and biodistribution

pattern in CT-26 tumor-bearing mice model. The estimated radiation doses to tumor and normal tissues for ^{177}Lu-labeled liposomes were about fourfolds higher than that for ^{111}In-labeled liposomes. The results suggested that i.v. administration of ^{111}In/^{177}Lu-labeled pegylated liposomes could lead to an adequate delivery of radiation dose to a solid tumor.

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