Pharmacokinetics and Dosimetry of $^{111}\text{In}/^{188}\text{Re}$ -Labeled PEGylated Liposomal Drugs in Two Colon Carcinoma-Bearing Mouse Models

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Abstract

PEGylated liposomes are important drug carriers for nanomedicine cancer therapy. PEGylated liposomes can encapsulate radio- and chemo-drugs and passively target tumor sites via enhanced permeability and retention effect. This study estimated the pharmacokinetics and dosimetry after administration of radio-chemotherapeutics (^{111}In -labeled vinorelbine [VNB]-encapsulated liposomes, InVNBL, and ^{188}Re -labeled doxorubicin [DXR]-encapsulated liposomes, ReDXRL) for radionuclide therapy in two colon carcinoma-bearing mouse models. A C26 colon carcinoma tumor/ascites mouse model and a subcutaneous solid tumor-bearing mouse model were employed. Biodistribution studies of InVNBL and ReDXRL after intraperitoneal administration in tumor/ascites-bearing mice (protocol A) and intravenous administration in subcutaneous solid tumor-bearing mice (protocol B) were performed. The radiation dose to normal tissues and tumors were calculated based on the results of distribution studies in mice, using the OLINDA/EXM program. The cumulated activities in most organs after administration of InVNBL in either the tumor/ascites-bearing mice (protocol A) or the subcutaneous solid tumor-bearing mice (protocol B) were higher than those of ReDXRL. Higher tumor-to-normal-tissues absorption dose ratios (T/NTs) were observed after administration of InVNBL than those of ReDXRL for protocol A. The T/NTs for the liver, spleen, and red marrow after injection of InVNBL for protocol B were similar to those of ReDXRL. The critical organ was found to be red marrow, and thus the red marrow absorption dose defined the recommended maximum administration activity of these liposomal drugs. Characterization of pharmacokinetics and dosimetry is needed to select the appropriate radiotherapeutics for specific tumor treatment applications. The results suggest that InVNBL is a promising therapeutic agent, which is as good as ReDXRL, in two mouse tumor models.

Key words: C26, dosimetry, doxorubicin, PEGylated liposomes, radionuclide therapy, vinorelbine

Introduction

Colorectal carcinoma is a common malignancy with poor prognosis. Invasion of colorectal carcinoma into the peritoneum induces malignant ascites, which is experienced

frequently in patients with advanced colorectal carcinoma and metastasis.^{1,2} The accumulation of drugs that causes local damage in tumor plays an important role in cancer therapy. However, the efficacy of employing only chemo- or radionuclide therapy was often limited because of normal tissue

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toxicity or high radiation burden in normal organs. The strategy of combined chemo-radiotherapy may achieve better tumor treatment efficacy and lower toxicity to normal tissues.³

PEGylated liposomes are capable of carrying drugs and providing passive targeting to tumors by means of enhanced permeability and retention (EPR) through leaky tumor vasculatures.^{4,5} Chemodrugs encapsulated in PEGylated liposomes, such as Lipo-Dox®, are a new generation of anticancer drugs and have been demonstrated with more favorable pharmacokinetic profiles, less cytotoxicity and enhanced antitumor activity.⁶ A new approach to combined chemotherapy and radionuclide cancer therapy involves the use of liposomes as drug delivery system.^{7,8} A previous study has demonstrated that PEGylated liposomes, containing radionuclide alone (¹⁸⁸Re-liposomes) or encapsulated with both radio- and chemodrugs (¹⁸⁸Re-DXR-liposomes), showed different biodistribution profiles and radiation dosimetry.⁹ In the present study, the *in vivo* radiation dosimetry of two chemodrug-encapsulated liposomes (Lipo-Dox and NanoVNB) labeled with Re-188 and In-111, respectively, have been investigated and compared.

Intraperitoneal (i.p.) administration of anticancer drugs and/or particles-emitting radionuclides-containing PEGylated liposomes is a better approach than intravenous (i.v.) administration in the treatment of peritoneal malignant cancer.¹⁰ The use of PEGylated liposomes with i.p. administration has been described in the treatment of ovarian cancer, leukemia, and lymphoma in preclinical and clinical studies.^{11–13} Radiolabeled liposomes are also widely studied to treat solid tumor via i.v. injection in mouse models.^{7,14,15}

Re-188, emitting 764 keV β -particles (mean energy) as well as a 155 keV gamma photon for scintigraphic imaging with a mean tissue penetration range of 2.43 mm and a physical half-life of 16.9 hours (Table 1), is useful for radionuclide therapy.¹⁶ ¹⁸⁸Re-labeled particulate nanomedicine, when retained in tumor owing to EPR, can kill tumor cells in close proximity to neovasculature by means of the strong crossfire effect. In-111 is a radionuclide commonly used for scintigraphic imaging ($t_{1/2}$: 2.81 days, 172 and 247 keV photons emission), emits 14.7 Auger electrons (mean energy: 0.46 keV) in average per decay (Table 1),¹⁷ and may also be suitable for radiotherapy. Auger electron, with an average energy of <2 keV, is a high LET radiation with subcellular path length (2–500 nm) in tissues. Auger electrons-emitting radiopharmaceuticals are highly toxic to cells when internalized into the cytoplasm, especially if incorporated into DNA, may cause chromosome damage, and has been suggested as radiotherapy agents for tumors.^{14,15,18}

The evaluation of safety and anticancer efficacy of internal emitters is important for radionuclide therapy.¹⁹ Many investigators and some regulatory agencies (e.g., the U.S. Food

and Drug Administration [FDA]) have used animal models for dosimetry calculations and for the establishment of treatment protocols in clinical trials.²⁰ Although one should never assume that a specific radiopharmaceutical will have identical pharmacokinetics in both animals and humans, the extrapolation of animal data to humans for dosimetric evaluation is advised and is usually performed for submission of an application for approval to the FDA for investigational new drug (IND) applications.¹⁶ Further, evaluation of the maximal tolerance doses (MTD) of drugs for patients is helpful to prescribe optimal approaches to therapy.

For evaluating the MTD of radiolabeled liposomes, finding the critical organ for radionuclide therapy is a prerequisite. In previous studies, hematologic toxicity in bone marrow after clinical trials of radioimmunotherapy and high liver uptake of PEGylated liposomes in animal studies have been observed.^{21,22} Either bone marrow or liver may be the dose-limiting organ for PEGylated liposomal radionuclide therapy.²¹ If the definition of tolerance is set at a too low level or is associated with a high degree of uncertainty, then patients may be systematically undertreated and potential therapeutic benefits will be decreased.

The present study evaluates and compares the pharmacokinetics and dosimetry of two radionuclide- and chemodrug-encapsulated liposomes (¹¹¹In-labeled vinorelbine [VNB]-encapsulated liposomes, InVNBL, and ¹⁸⁸Re-labeled doxorubicin [DXR]-encapsulated liposomes, ReDXRL) in a C26 colon carcinoma tumor/ascites-bearing mouse model and a subcutaneous solid tumor-bearing mouse model. The absorption doses of critical organs and tumors were estimated on the basis of mouse biodistribution studies, using the OLINDA/EXM software package.²³ The tumor-to-normal-tissues absorption dose ratios (T/NTs) of InVNBL were compared with those of ReDXRL in these two animal tumor models. The maximum administration activity for conducting radionuclide therapy on human subjects was estimated based on the biokinetics observed in this study.

Materials and Methods

Preparation of liposomes

Lipo-Dox® (Lipo-Dox) was purchased from Taiwan Tung Yang Biopharm (TTY Biopharm Company Ltd., Taipei, Taiwan). Lipo-Dox contains 2 mg/mL of doxorubicin and 14 μ mol/mL phospholipid. Its lipid compositions include distearoylphosphatidylcholine (DSPC), cholesterol, and polyethylene glycol (average MW: 2000)-derived distearoylphosphatidylethanolamine (PEG-DSPE) (molar ratio: 3:2:0.3). The preparation of Lipo-Dox and labeling method for ¹⁸⁸Re-BMEDA and ¹⁸⁸Re-liposomes have been previously described.^{24,25} Lipo-Dox (1 mL) was added to the ¹⁸⁸Re-BMEDA (50–74 MBq) and incubated at 60°C for 30 minutes.

TABLE 1. RADIONUCLIDE PROPERTIES

Radionuclide	Half-life (day)	Mean $E_{particle}$ (keV)	$E_{photons}$ (keV)	Tissue penetration R_{approx}
Re-188	0.71	764 (β)	155 (15%)	2.43 mm
In-111	2.81	0.46 (14.7 Auger e)	172 (89%), 247 (94%)	2–500 nm

E, energy; *R*, range.

The ReDXRL was separated from free ^{188}Re -BMEDA using a PD-10 column (GE Healthcare, Uppsala, Sweden) eluted with normal saline. The labeling efficiency was determined by dividing the radioactivity of Lipo-Dox fractions after separation by total radioactivity before separation.

For InVNBL, the PEGylated liposomes were prepared from DSPC, cholesterol, and PEG-DSPE (molar ratio: 3:2:0.045) and then encapsulated with vinorelbine (VNB, 2 mg/mL) to give NanoVNB, was obtained from Taiwan Liposome Company (Taipei, Taiwan). The labeling method for VNB-encapsulated NanoVNB with ^{111}In -oxine was detailed in a previous report.¹⁰ Briefly, ^{111}In -oxine residue was dissolved in 20 μL of ethanol, added with 80 μL of distilled water, and then incubated with 2 mL NanoVNB for 30 minutes at 37°C. About 100 μL of reaction solution was loaded on to a column (40 $\times$ 8 mm; Bio-Rad; Hercules, CA) containing SephadexTM G-50 Fine gel and eluted with normal saline. The labeling efficiency was determined by dividing the radioactivity of the NanoVNB fractions after separation by total radioactivity before separation. The particle size of ReDXRL and InVNBL (after decay to background) was determined using dynamic laser scattering with a submicron particle analyzer (N4 Plus, Beckman Coulter). The labeling yield and the radiochemical purity of ReDXRL and InVNBL were all greater than 90%. The average particle size of ReDXRL and InVNBL was 131 $\pm$ 30 and 102 $\pm$ 6.9 nm, which was similar to that of Lipo-Dox (118 $\pm$ 44 nm) and NanoVNB (95.2 $\pm$ 4.9 nm).

Cell Line and animal tumor models

The establishment of C26 colon adenocarcinoma tumor/ascites-bearing mouse model and subcutaneous solid tumor-bearing mouse model has been previously reported.^{24,25} Briefly, for the mouse tumor/ascites model, male BALB/c mice (6–8 weeks old) were inoculated i.p. with 2×10^5 C26 cells in 500 μL phosphate-buffered saline. Animal studies were conducted at 10 days after inoculation. For the solid tumor-bearing mouse model, each mouse was subcutaneously inoculated with 2×10^5 C26 cells in the right hind flank. Two (2) weeks later, the tumor xenograft grew to about 100 mm³ and the mouse was ready for animal study. All animal protocols were approved by the Institutional Animal Care and Use Committee at National Yang-Ming University (Taipei, Taiwan) and the Institute of Nuclear Energy Research (Taoyuan, Taiwan).

Pharmacokinetic studies

For the mouse tumor/ascites model, each animal ($n = 4$ for each time point) received intraperitoneal injection of 1.85 MBq/100 μL of ReDXRL with 0.47 μmol phospholipids or 3.7 MBq/100 μL of InVNBL with 0.18 μmol phospholipids (protocol A). The mice were sacrificed by CO₂ asphyxiation at 1, 4, 24, 48, and 72 hours postinjection. For the solid tumor-bearing mouse model, each animal ($n = 4$ for each time point) was intravenously injected with 1.85 MBq/100 μL of ReDXRL (0.17 μmol phospholipid) or 3.7 MBq/100 μL of InVNBL (0.18 μmol phospholipids) (protocol B). Mice were sacrificed at 1, 4, 24, 48, and 72 hours by CO₂ asphyxiation. Data were decay corrected and expressed as percentage of the injected activity per gram of tissue (%IA/g). The area under the curve (AUC), which corresponds to the integral of

the amount of disintegrations ($\text{h} \times \% \text{IA/g}$), was calculated for important source organs.

Radiation dose calculations

For the estimation of absorption doses in each organ and total body of human, the relative organ mass scaling method was used.^{26,27} The uptake and dose in various tissues/organs were derived from the radioactivity concentration in tissues and organs of interest, assuming a homogeneous distribution within each source region.²⁰ The calculated mean value of %IA/g for the organs in mice was extrapolated to uptake in organs of a 70-kg adult using the following formula²⁶:

$$\left[\left(\frac{\% \text{IA}}{g_{\text{organ}}} \right)_{\text{animal}} \times (kg_{\text{TB weight}})_{\text{animal}} \right] \times \left(\frac{g_{\text{organ}}}{kg_{\text{TB weight}}} \right)_{\text{human}} = \left(\frac{\% \text{IA}}{\text{organ}} \right)_{\text{human}}$$

The extrapolated values (%IA) in the human organs at 1, 4, 24, 48, and 72 hours were fitted with exponential functions and integrated to obtain the number of disintegrations in the source organs; this information was input into the OLINDA/EXM computer program. The integrals (MBq-h/MBq administered) for 14 organs, including heart contents (blood), brain, muscle, bone, heart, lung, testes, spleen, pancreas, kidneys, liver, stomach, small intestine, lower intestine, and the remainder of the body, were calculated and used for the dosimetry estimation.

To estimate the absorption doses of tumors of various mass ranging from 0.5 to 300 g, the mean tumor uptake (%IA/g) obtained in the biodistribution studies (Tables 2 and 3) was directly fitted with exponential models to calculate the number of disintegrations, which again was input to the OLINDA/EXM computer program using the unit density sphere model (used to estimate doses to isolated tumors, animal organs, or other objects assumed to be approximately spherical and to have a uniform activity distribution, and only self-dose is calculated).²³ The T/NTs were then calculated from the mean dose of a 300-g tumor and normal tissues (liver, spleen, and red marrow).

Results

Pharmacokinetic studies and tissue radiation dose calculations

The pattern of radioactivity distribution in tissues represented the pharmacokinetics of these liposomal drugs *in vivo*. The radioactivities in those organs that accumulate significant amounts of i.p. and i.v. administered liposomal drugs (the critical organs) are shown in Table 2 for the mouse tumor/ascites model and in Table 3 for the solid tumor-bearing mouse model. The AUCs of tumor and ascites after InVNBL injection in the mouse tumor/ascites model (protocol A) were higher than those after ReDXRL injection (5.4-fold and 1.3-fold; Table 4). The cumulated radioactivity retained in the normal tissues (e.g., blood and spleen) of InVNBL-injected mice was somewhat less than those of the ReDXRL group. The AUCs of all critical organs as well as the tumor uptake in the solid tumor-bearing mice

TABLE 2. TUMOR AND ORGAN UPTAKES AFTER INTRAPERITONEAL ADMINISTRATION OF InVNBL AND ReDXRL IN C26 COLON CARCINOMA TUMOR/ASCITES-BEARING MOUSE MODEL

Organ	1 hour	4 hours	24 hours	48 hours	72 hours
Tumor	12.6 ± 4.45 (i) 1.79 ± 0.32 (ii)	14.7 ± 5.36 2.63 ± 0.39	17.1 ± 1.41 3.49 ± 1.56	20.4 ± 1.07 2.74 ± 0.20	11.5 ± 2.99 3.62 ± 0.87
Blood	10.4 ± 4.31 (i) 4.84 ± 0.60 (ii)	8.02 ± 6.08 8.61 ± 1.14	2.05 ± 0.67 8.21 ± 1.79	0.79 ± 0.50 7.16 ± 1.45	0.70 ± 0.41 3.64 ± 0.42
Liver	3.04 ± 0.16 (i) 1.90 ± 0.10 (ii)	3.71 ± 0.68 2.88 ± 0.42	5.78 ± 0.89 5.56 ± 1.33	9.54 ± 1.62 5.84 ± 0.65	19.8 ± 1.31 4.84 ± 0.35
Spleen	4.14 ± 0.41 (i) 1.80 ± 0.34 (ii)	4.55 ± 1.15 3.80 ± 1.11	6.23 ± 0.23 6.87 ± 1.37	6.64 ± 1.39 27.8 ± 6.14	18.6 ± 4.62 24.3 ± 4.05
Bone	0.79 ± 0.04 (i) 0.61 ± 0.13 (ii)	0.92 ± 0.30 1.01 ± 0.18	2.42 ± 0.64 0.77 ± 0.16	3.84 ± 0.49 1.25 ± 0.27	4.47 ± 0.21 0.69 ± 0.18
Ascites	179 ± 8.36 (i) 126 ± 9.49 (ii)	94.4 ± 4.01 57.4 ± 13.2	19.7 ± 0.96 20.5 ± 2.88	16.9 ± 0.85 15.2 ± 2.52	8.67 ± 2.95 5.89 ± 0.79

Values were decay corrected and expressed as %IA/g, mean ± SEM ($n=4$ at each time point). (i): InVNBL; (ii): ReDXRL.

after InVNBL injection (protocol B) were higher than those after ReDXRL injection (2.2–12.0-fold and 7.2-fold, respectively).

The calculated doses in important target organs are shown in Table 5. The absorption doses for most critical normal organs after InVNBL administration were slightly higher than those after ReDXRL administration for protocol A, except for the spleen. Higher doses for normal organs after InVNBL administration compared with those after ReDXRL administration were also observed for protocol B. The liver and spleen received the highest doses, several times higher than the other organs. As was observed in this study, administration of InVNBL and ReDXRL via i.v. route (protocol B) resulted in higher normal tissues doses than those via i.p. route (protocol A).

Tumor radiation dose calculations

After administration of liposomal drugs in a mouse tumor/ascites model, the calculated tumor dose (assuming a mass of 300 g) was 2.3 mGy/MBq (i.p. injection, InVNBL) and 1.1 mGy/MBq (i.p. injection, ReDXRL). In the solid tumor-bearing mouse model, the calculated dose to tumor (also assuming a mass of 300 g) was 2.1 mGy/MBq (i.v. injection, InVNBL) and 1.0 mGy/MBq (i.v. injection, ReDXRL), respectively (Table 6).

In the mouse tumor/ascites model, all the T/NTs after i.p. administration of InVNBL were higher than those of ReDXRL (protocol A). In the solid tumor-bearing mouse model, the tumor-to-spleen (T/S) and tumor-to-red marrow (T/RM) ratios for i.v. administration of InVNBL were higher than those of ReDXRL (protocol B), except for tumor-to-liver (T/LV) ratio.

Discussion

Liposomes have been investigated widely as universal carriers of tumor chemotherapeutic agents, as antigen carriers to stimulate immune response, as carriers of nucleic acid for gene therapy, and as carriers of antibiotics for infectious disease treatment.^{5,28} Several liposome-encapsulated radionuclides have been used for internal radiotherapy.^{21,29} Among these, In-111 and Re-188 are regarded as favorable radionuclides from the standpoint of pharmacokinetics and dosimetry and because they have imageable photons.^{10,15,18,24,25,29,30} Radionuclide selection involves the physical half-life and the emission property of the radionuclide, which are put together to optimize the therapeutic effect (Table 1). Considering the time needed for liposomal drugs to reach maximum tumor uptake (1–2 days postinjection; Tables 2 and 3), the longer half-life of In-111 (2.82 days) would be better than that of Re-188 (0.71 days). The

TABLE 3. TUMOR AND ORGAN UPTAKES AFTER I.V. ADMINISTRATION OF InVNBL AND ReDXRL IN C26 COLON CARCINOMA SOLID TUMOR-BEARING MOUSE MODEL

Organ	1 hour	4 hours	24 hours	48 hours	72 hours
Tumor	4.37 ± 0.79 (i) 1.68 ± 0.08 (ii)	4.47 ± 0.91 3.08 ± 0.53	25.3 ± 1.99 3.19 ± 0.50	19.1 ± 4.86 2.22 ± 0.19	15.8 ± 1.50 1.06 ± 0.30
Blood	49.9 ± 6.69 (i) 28.2 ± 0.60 (ii)	44.1 ± 2.81 17.4 ± 0.58	7.72 ± 1.04 4.34 ± 0.17	5.42 ± 0.77 2.40 ± 0.11	4.70 ± 0.67 1.57 ± 0.05
Liver	13.5 ± 0.45 (i) 11.1 ± 0.81 (ii)	14.5 ± 0.19 11.5 ± 0.41	46.1 ± 2.65 5.07 ± 0.48	42.3 ± 1.75 2.82 ± 0.57	42.9 ± 4.08 2.48 ± 0.11
Spleen	11.8 ± 0.83 (i) 9.39 ± 0.66 (ii)	17.9 ± 0.63 14.7 ± 3.37	61.6 ± 3.46 7.80 ± 1.92	64.6 ± 4.62 3.70 ± 0.52	56.8 ± 4.32 2.50 ± 0.17
Bone	2.93 ± 0.18 (i) 1.46 ± 0.33 (ii)	3.01 ± 0.75 0.90 ± 0.09	6.02 ± 0.21 0.50 ± 0.04	6.79 ± 0.44 0.31 ± 0.03	5.78 ± 0.12 0.12 ± 0.01

Values were decay corrected and expressed as %IA/g, mean ± SEM ($n=4$ at each time point). (i): InVNBL; (ii): ReDXRL.

TABLE 4. AREAS UNDER THE CURVE AND RELATIVE RATIO OF AREAS UNDER THE CURVE OF TISSUES POSTADMINISTRATION OF LIPOSOMAL DRUGS IN COLON CARCINOMA MOUSE MODELS VIA TREATMENT PROTOCOLS A AND B

Tissue	AUC				RR-AUC	
	Protocol A		Protocol B		InVNBL vs. ReDXRL	
	InVNBL	ReDXRL	InVNBL	ReDXRL	Protocol A	Protocol B
Tumor	1198	220	1267	176	5.4	7.2
Blood	185	512	989	445	0.4	2.2
Liver	642	357	2745	369	1.8	7.4
Spleen	580	1157	3823	483	0.5	7.9
Bone	211	68.1	407	34.0	3.1	12.0
Ascites	2387	1799	—	—	1.3	—

AUC values were derived by mean value of biodistribution studies ($h \times \%IA/g$, $n=4$ at each time point).

AUC, area under the curve; RRAUC, relative ratio of AUC.

tumors in the two mouse models receive significantly higher cumulated activity after administration of InVNBL (5.4-fold and 7.2-fold) than that of ReDXRL (Table 4). However, there was a difference in pharmacokinetics between protocol A and protocol B. In the solid tumor mouse model (protocol B), high initial radioactivity in the blood after i.v injection of liposomal drugs may account for the significant drug accumulation in the RES organs (liver and spleen) because of the high first-pass uptake through the liver. In the tumor/ascites mouse model (protocol A), the liposomal drugs were injected into the intraperitoneal cavity (containing ascites and tumor); the liposomal drugs then slowly transported back to the blood circulation and resulted in lower radioactivity accumulation in the normal tissues (e.g., liver and spleen) than those administered in protocol B.

The degree of PEGylation may also influence the stability and pharmacokinetic profiles of liposomes *in vivo*. As suggested by Li and Huang, a concentration of 5 mol% PEG may be an optimal level for PEGylated liposomal nanoparticles.³¹ Excessive PEGylation might disturb the balance of hydrophilicity and hydrophobicity by disrupting the integrity of the surface lipid bilayer.³¹ A low level of PEGylation would result in higher reticuloendothelial system (RES) uptake.³² In the present study, a concentration of 0.9 mol% PEG of InVNBL demonstrated higher liver, spleen, and bone uptakes than those of 6 mol% PEG of ReDXRL after i.v. injection in the solid tumor-bearing mouse model (Table 4), which is consistent with the findings of Chow et al.³² Although high

uptakes in critical organs were observed after administration of InVNBL and ReDXRL, it has been previously demonstrated that they resulted in acceptable toxicity in mice. The histopathologic results showed regular morphology of the liver, spleen, and kidney of mice and no significant damage was observed.^{7,15,18} In future investigations, lower critical organ accumulation may be achieved by applying various strategies in liposome composition, including suitable PEGylation (5–10 mol% PEG) of the liposomal surface, optimal particle size (~ 100 nm diameter), and neutral lipid structure (± 10 mV of zeta potential). Other formulations such as receptor-mediated immunoliposomes, pH-sensitive liposomes, and thermal-sensitive liposomes have also been reported to be useful. In tumor treatment, prior administration of empty liposomes to occupy the RES-rich organs has also been employed to improve the bioavailability of nanomedicine.

It is theoretically difficult to perform effective tumor therapy with radionuclides that emit very low-energy electrons, if the tumor cells are not targeted because of the lack of crossfire effect.^{33,34} In-111, a short-range Auger electron emitter, delivers inhomogeneous dose distributions in tumors when the radionuclide distribution in the tumor is not uniform.¹⁴ The passive targeting PEGylated liposomes were designed to overcome this problem. Liposomes are regarded as an effective delivery system for radionuclide therapy by disposing an almost uniform absorption dose profile at the central regions of micrometastatic tumors.¹⁹ In the present study, high tumor accumulation ($1198 h \times \%IA/g$, mouse

TABLE 5. ESTIMATED RADIATION ABSORPTION DOSES (MGY/MBQ) OF TISSUES POSTADMINISTRATION OF LIPOSOMAL DRUGS IN COLON CARCINOMA MOUSE MODELS VIA TREATMENT PROTOCOLS A AND B

Tissue	Protocol A		Protocol B	
	InVNBL	ReDXRL	InVNBL	ReDXRL
Liver	0.20	0.17	0.61	0.26
Spleen	0.16	0.36	0.57	0.32
Kidneys	0.14	0.08	0.24	0.13
Red marrow	0.092	0.056	0.11	0.053
Total body	0.095	0.090	0.12	0.090

Extrapolated radiation absorption dose for a 70-kg male adult.

TABLE 6. TUMOR-TO-NORMAL-TISSUE ABSORPTION DOSE RATIOS FOR INVNBL AND REDXRL IN COLON CARCINOMA MOUSE MODELS VIA TREATMENT PROTOCOLS A AND B

	Protocol A		Protocol B	
	InVNBL	ReDXRL	InVNBL	ReDXRL
Tumor	2.3	1.1	2.1	1.0
T/LV	11	6.5	3.4	3.8
T/S	14	3.1	3.7	3.1
T/RM	25	20	20	19

Tumor-to-normal-tissues absorption dose ratios were calculated from mean dose in a 300-g tumor and tissues.

T, tumor; LV, liver; S, spleen; RM, red marrow.

tumor/ascites model; 1267 h $\times$ %IA/g, solid tumor-bearing mouse model) was observed after InVNBL injection.

In-111 could also be used as the chemical and biological surrogate of Y-90, which has a physical half-life (2.67 days) similar to In-111. The radiation dosimetry for Y-90 could be projected based on the assumption of identical biologic behavior of ^{111}In - and ^{90}Y -labeled molecules.²⁹ Y-90, with its relatively long-range β -particle emissions would minimize the nonuniformity associated with the dose distribution. For future clinical study, the potential of ^{90}Y -labeled liposomes as an anticancer drug for radionuclide therapy could be assessed by examining the projected radiation doses on the basis of the quantitative uptake parameters found from the images of ^{111}In -labeled liposomes.

In this study, the absorption doses of the liver, red marrow, and total body were 0.61, 0.11, and 0.12 mGy/MBq for InVNBL and 0.26, 0.053, and 0.090 mGy/MBq for ReDXRL (i.v. injection, solid tumor animal model; Table 5). These doses are lower than those reported by Emfietzoglou et al., which were 1.8, 0.11 and 0.50 mGy/MBq for the ^{90}Y -labeled SUV-liposomes and 0.44, 0.12, and 0.15 mGy/MBq for the ^{188}Re -labeled SUV-liposomes, respectively.²¹ The liposome formulations used in the present study (NanoVNB and LipoDox), which were also used in clinical studies, provided a better pharmacokinetic profile than that reported by Emfietzoglou et al. Radionuclide therapy employing PEGylated liposome as a passive targeting carrier, compared with those using direct radionuclide-labeled substrates, peptides, or monoclonal antibodies in radioimmunotherapy, may have the benefit of reduced dose and toxicity in normal organs. Pandit-Taskar et al. reported liver and red marrow doses of 0.75 and 0.11 mGy/MBq, respectively, after injection of ^{111}In -huJ591, which were somewhat higher than those observed in the present study (0.61 and 0.11 mGy/MBq for InVNBL in the solid tumor-bearing mice).³⁵ Higher doses for total body and liver after injection of ^{111}In -DTPA-hEGF (0.19 and 0.76 mGy/MBq, respectively) compared with those of InVNBL and ReDXRL were also observed.³⁶ The absorption doses to the spleen, liver, kidney, and red marrow after injection of InVNBL and ReDXRL in this study were lower than those reported in clinical trials using ^{90}Y -DOTATOC (1.5–19.4, 0.1–2.6, 1.1–10.3, and 0.01–0.2 mGy/MBq, respectively) for peptide receptor radionuclide therapy of cancer patients.³⁷

For tumor dose calculations, it was assumed that the absorption doses to tumors with different weights were due to only a mass effect. The highest tumor dose was 2.3 mGy/MBq after i.p. administration of InVNBL in mouse tumor/ascites model (protocol A). The T/NTs, including the liver, spleen, and red marrow, were approximately 11, 14, and 25 after injection of InVNBL for protocol A, which were higher than those of ReDXRL (6.5, 3.1, and 20), respectively (Table 6). The T/NTs for the liver, spleen, and red marrow after i.v. injection of InVNBL (3.4, 3.7 and 20) in solid tumor-bearing mice (protocol B) were similar to those of ReDXRL (3.8, 3.1 and 19). More significant differences in the T/NTs after administration of InVNBL and ReDXRL in protocol A than in protocol B were observed. The short penetration range of In-111 in tissues renders it fitter to work against the tumor metastasis and ascites in abdomen than Re-188 does (protocol A). The present study indicated that InVNBL may be a more promising agent than ReDXRL for tumor treat-

ment via i.p. administration in the mouse tumor/ascites model. For protocol B, in which the liposomal drugs were injected intravenously in a solid tumor-bearing mouse model, ReDXRL is potentially as effective a therapeutic agent as InVNBL.

The OLINDA/EXM code gives estimates of dose for specific standardized human models of adults and children of different weights. Data extrapolated from animal species can be thus used to obtain dose estimates for humans. Conventional external beam radiotherapy commonly adopts dose fractions of about 2 Gy in the tumor, with doses delivered 5 days per week over a period of a few weeks, to a total dose of 50–70 Gy, depending on tumor type and size.²⁹ For the radiolabeled PEGylated liposome delivery systems, a comparable radiation dose to the tumor may be achieved by repeated administrations of these liposomal drugs. Such an approach, with multiple administrations to control the absorption doses to nontarget tissues, like usage of Bexxar (a radioimmunotherapeutic agent approved by the U.S. FDA for treatment of non-Hodgkin's lymphoma),^{38,39} may be applied for InVNBL and ReDXRL in future clinical trials.

The tolerance dose (TD) in the liver is 30 Gy with whole organ in external beam therapy, defined as the dose for a probability of 5% complication in 5 years (TD 5/5).⁴⁰ However, dose rate effects are important in radionuclide therapy because of the ability to produce differential sparing effects between normal and malignant tissue. Biologically effective dose (BED) calculations allow for the quantitative assessment of the biological effects associated with different dose rates and this should be considered in future study.⁴¹ The tolerance dose in red marrow of 1.85 Gy was chosen, representing a probability of major platelet toxicity of approximately 30%.²² Bone marrow toxicity is generally dose-limiting for radioimmunotherapy with β -emitting radiopharmaceuticals; using the operational definitions of maximum tolerance dose defined earlier (1.85 Gy), the MAA was 6.7 GBq (180 mCi) for administration of an ^{131}I -labeled antibody.²² When red marrow was chosen as the critical organ, the MAA of the InVNBL and ReDXRL were 20 and 33 GBq for protocol A and 17 and 35 GBq for protocol B, respectively, in the present study. Generally, radiation therapy that delivers a higher absorption dose to tumor produces greater tumor response. After injection of InVNBL and ReDXRL with MAAs, the tumor absorption doses were 46 and 36 Gy for protocol A and 36 and 35 Gy for protocol B, respectively. At the same time, the liver doses for both InVNBL (4.0 Gy, protocol A; 10.5 Gy, protocol B) and ReDXRL treatments (5.6 Gy, protocol A; 9.1 Gy, protocol B) were much lower than the maximum tolerance dose of the liver (30 Gy).⁴⁰

To the best of the authors' knowledge, the present study is the first to estimate the absorption dose to tumor and normal tissues after administration of ^{111}In -labeled PEGylated liposomes in a mouse tumor/ascites model and a solid tumor-bearing mouse model. To correctly estimate the absorption doses in human subjects, using OLINDA/EXM program to calculate organ doses based on the biodistribution study of animals is the first step. More accurate human dosimetry must be established with imaging studies involving human volunteers or patients. The dosimetry study presented here will be useful in the planning of these studies and for the application of InVNBL and ReDXRL for IND research.

Conclusions

Characterization of pharmacokinetics and radiation dosimetry is important in selecting the appropriate radiotherapeutics for specific tumor therapy applications. Radionuclide therapy employing a PEGylated liposome may have the benefit of reduced dose and toxicity in normal organs. The results suggest that InVNBL is a promising therapeutic agent as good as, or better than, ReDXRL when administered in these two colon carcinoma mouse tumor models. Red marrow is the critical organ in determining the MAA of PEGylated liposomal drugs.

Acknowledgments

The authors thank Dr. Tsai-Yueh Luo for her help with the preparation of ^{188}Re . This study was supported by grants from the National Health Research Institutes, Miaoli, Taiwan (96A1-NMPP01-007 and 97A1-NMPP01-007) and Department of Health, Taipei City Government, Taiwan (98001-62-029 and 99001-62-034). The NanoVNB liposomes were kindly provided by Taiwan Liposome Company.

Disclosure Statement

No competing financial interests exist.

References

1. Markman M. Intraperitoneal chemotherapy in the management of colon cancer. *Semin Oncol* 1999;26:536.
2. Kanellos I, Demetriades H, Zintzaras E, et al. Incidence and prognostic value of positive peritoneal cytology in colorectal cancer. *Dis Colon Rectum* 2003;46:535.
3. Dancey JE, Chen HX. Strategies for optimizing combinations of molecularly targeted anticancer agents. *Nat Rev Drug Discov* 2006;5:649.
4. Allen TM, Cullis PR. Drug delivery systems: entering the mainstream. *Science* 2004;303:1818.
5. Torchilin VP. Recent advances with liposomes as pharmaceutical carriers. *Nat Rev Drug Discov* 2005;4:145.
6. Gabizon A, Shmeeda H, Barenholz Y. Pharmacokinetics of pegylated liposomal Doxorubicin: review of animal and human studies. *Clin Pharmacokinet* 2003;42:419.
7. Chang YJ, Chang CH, Yu CY, et al. Therapeutic efficacy and microSPECT/CT imaging of ^{188}Re -DXR-liposome in a C26 murine colon carcinoma solid tumor model. *Nucl Med Biol* 2010;37:95.
8. Chen LC, Chang CH, Yu CY, et al. Pharmacokinetics, microSPECT/CT imaging and therapeutic efficacy of ^{188}Re -DXR-liposome in C26 colon carcinoma ascites mice model. *Nucl Med Biol* 2008;35:883.
9. Chang CH, Stabin MG, Chang YJ, et al. Comparative dosimetric evaluation of nanotargeted ^{188}Re -(DXR)-liposome for internal radiotherapy. *Cancer Biother Radiopharm* 2008;23:749.
10. Lin YY, Li JJ, Chang CH, et al. Evaluation of pharmacokinetics of ^{111}In -labeled VNB-PEGylated liposomes after intraperitoneal and intravenous administration in a tumor/ascites mouse model. *Cancer Biother Radiopharm* 2009;24:453.
11. Sadzuka Y, Hirota S, Sonobe T. Intraperitoneal administration of doxorubicin encapsulating liposomes against peritoneal dissemination. *Toxicol Lett* 2000;116:51.
12. Cabanes A, Tzemach D, Goren D, et al. Comparative study of the antitumor activity of free doxorubicin and polyethylene glycol-coated liposomal doxorubicin in a mouse lymphoma model. *Clin Cancer Res* 1998;4:499.
13. Vaage J, Donovan D, Mayhew E, et al. Therapy of human ovarian carcinoma xenografts using doxorubicin encapsulated in sterically stabilized liposomes. *Cancer* 1993;72:3671.
14. Uusijarvi H, Bernhardt P, Ericsson T, et al. Dosimetric characterization of radionuclides for systemic tumor therapy: influence of particle range, photon emission, and subcellular distribution. *Med Phys* 2006;33:3260.
15. Chow TH, Lin YY, Hwang JJ, et al. Therapeutic efficacy evaluation of ^{111}In -labeled PEGylated liposomal vinorelbine in murine colon carcinoma with multimodalities of molecular imaging. *J Nucl Med* 2009;50:2073.
16. Milenic DE, Brady ED, Brechbiel MW. Antibody-targeted radiation cancer therapy. *Nat Rev Drug Discov* 2004;3:488.
17. Kereiakes JG, Rao DV. Auger electron dosimetry: report of AAPM Nuclear Medicine Committee Task Group No. 6. *Med Phys* 1992;19:1359.
18. Chow TH, Lin YY, Hwang JJ, et al. Diagnostic and therapeutic evaluation of ^{111}In -vinorelbine-liposomes in a human colorectal carcinoma HT-29/luc-bearing animal model. *Nucl Med Biol* 2008;35:623.
19. Sgouros G. Dosimetry of internal emitters. *J Nucl Med* 2005;46 Suppl 1:18S.
20. Measurements ICoRUa. Absorbed-Dose Specification in Nuclear Medicine, ICRU Report 67. *J ICRU* 2002;2:15.
21. Emfietzoglou D, Kostarelos K, Sgouros G. An analytic dosimetry study for the use of radionuclide-liposome conjugates in internal radiotherapy. *J Nucl Med* 2001;42:499.
22. O'Donoghue JA, Baidoo N, Deland D, et al. Hematologic toxicity in radioimmunotherapy: dose-response relationships for I-131 labeled antibody therapy. *Cancer Biother Radiopharm* 2002;17:435.
23. Stabin MG, Sparks RB, Crowe E. OLINDA/EXM: the second-generation personal computer software for internal dose assessment in nuclear medicine. *J Nucl Med* 2005;46:1023.
24. Chang YJ, Chang CH, Chang TJ, et al. Biodistribution, pharmacokinetics and MicroSPECT/CT imaging of ^{188}Re -BMEDA-liposome in a C26 murine colon carcinoma solid tumor animal model. *Anticancer Res* 2007;27:2217.
25. Chen LC, Chang CH, Yu CY, et al. Biodistribution, pharmacokinetics and imaging of ^{188}Re -BMEDA-labeled pegylated liposomes after intraperitoneal injection in a C26 colon carcinoma ascites mouse model. *Nucl Med Biol* 2007;34:415.
26. Stabin MG, Siegel JA. Physical models and dose factors for use in internal dose assessment. *Health Phys* 2003;85:294.
27. Molina-Trinidad EM, de Murphy CA, Ferro-Flores G, et al. Radiopharmacokinetic and dosimetric parameters of ^{188}Re -lanreotide in athymic mice with induced human cancer tumors. *Int J Pharm* 2006;310:125.
28. Bao A, Goins B, Klipper R, et al. ^{186}Re -liposome labeling using ^{186}Re -SNS/S complexes: *in vitro* stability, imaging, and biodistribution in rats. *J Nucl Med* 2003;44:1992.
29. Kostarelos K, Emfietzoglou D. Tissue dosimetry of liposome-radionuclide complexes for internal radiotherapy: toward liposome-targeted therapeutic radiopharmaceuticals. *Anticancer Res* 2000;20:3339.
30. Harrington KJ, Rowlinson-Busza G, Syrigos KN, et al. Influence of tumour size on uptake of ^{111}In -DTPA-labelled pegylated liposomes in a human tumour xenograft model. *Br J Cancer* 2000;83:684.
31. Li SD, Huang L. Pharmacokinetics and biodistribution of nanoparticles. *Mol Pharm* 2008;5:496.

32. Chow TH, Lin YY, Hwang JJ, et al. Improvement of bio-distribution and therapeutic index via increase of poly-ethylene glycol on drug-carrying liposomes in an HT-29/luc xenografted mouse model. *Anticancer Res* 2009;29:2111.
33. Bernhardt P, Ahlman H, Forssell-Aronsson E. Model of metastatic growth valuable for radionuclide therapy. *Med Phys* 2003;30:3227.
34. O'Donoghue JA. Implications of nonuniform tumor doses for radioimmunotherapy. *J Nucl Med* 1999;40:1337.
35. Pandit-Taskar N, O'Donoghue JA, Morris MJ, et al. Antibody mass escalation study in patients with castration-resistant prostate cancer using ^{111}In -J591: lesion detectability and dosimetric projections for ^{90}Y radioimmunotherapy. *J Nucl Med* 2008;49:1066.
36. Reilly RM, Chen P, Wang J, et al. Preclinical pharmacokinetic, biodistribution, toxicology, and dosimetry studies of ^{111}In -DTPA-human epidermal growth factor: An auger electron-emitting radiotherapeutic agent for epidermal growth factor receptor-positive breast cancer. *J Nucl Med* 2006;47:1023.
37. Cremonesi M, Ferrari M, Bodei L, et al. Dosimetry in Peptide radionuclide receptor therapy: a review. *J Nucl Med* 2006;47:1467.
38. Kaminski MS, Zasadny KR, Francis IR, et al. Iodine-131-anti-B1 radioimmunotherapy for B-cell lymphoma. *J Clin Oncol* 1996;14:1974.
39. Wahl RL. The clinical importance of dosimetry in radioimmunotherapy with tositumomab and iodine I 131 tositumomab. *Semin Oncol* 2003;30:31.
40. Emami B, Lyman J, Brown A, et al. Tolerance of normal tissue to therapeutic irradiation. *Int J Radiat Oncol Biol Phys* 1991;21:109.
41. Fowler JF. Radiobiological aspects of low dose rates in radioimmunotherapy. *Int J Radiat Oncol Biol Phys* 1990;18:1261.