SPANDIDOS PUBLICATIONS

ONCOLOGY REPORTS 27: 678-684, 2012

Comparison of the therapeutic efficacy of ^{188}Rhenium-liposomes and liposomal doxorubicin in a 4T1 murine orthotopic breast cancer model

CHI-MOU LIU[1], WAN-CHI LEE[1], CHIA-YU YU[1], KENG-LI LAN[2], CHIH-HSIEN CHANG[1], GANN TING[1] and TE-WEI LEE[1]

[1]Isotope Application Division, Institute of Nuclear Energy Research, No. 1000 Wenhua Road, Chiaan Village, Lungtan, Taoyuan 325; [2]Cancer Center, Taipei Veterans General Hospital, No. 201, Sec. 2, Shipai Road, Beitou District, Taipei 112, Taiwan, R.O.C.

Received August 30, 2011; Accepted October 1, 2011

DOI: 10.3892/or.2011.1557

Abstract. Liposomal doxorubicin (Lipo-DOX) has been widely and successfully used in chemotherapy for breast cancer patients. Since our previous studies found that ^{188}Rhenium (^{188}Re)-N,N-bis (2-mercaptoethyl)-N',N'-diethylethylenediamine (BMEDA)-labeled pegylated liposomes (^{188}Re-liposomes) have radiotherapeutic potential in a colon cancer model, and little information is available to make a comparison of the therapeutic efficacy of internal radiotherapy and chemotherapy, this study evaluates the therapeutic efficacy of ^{188}Re-liposomes and Lipo-DOX, in a 4T1 murine orthotopic breast cancer model. MicroSPECT/CT imaging showed that the highest uptake of ^{188}Re-liposomes was found at 24 h after intravenous administration. The results of a biodistribution assay also demonstrated that the highest uptake of ^{188}Re-liposomes in a tumor was 3.03 ± 0.29 (%ID/g) at 24 h, and that the highest tumor to muscle ratio was approximately 17 at 48 h. According to measurements of body weight and survival rate, the maximum tolerated doses (MTD) of ^{188}Re-liposomes and Lipo-DOX were 37 MBq and 25 mg/kg, respectively. In a study of therapeutic efficacy, mice with 4T1 orthotopic breast tumors that were treated with ^{188}Re-liposomes (4/5 MTD, 29.6 MBq) or Lipo-DOX (4/5 MTD, 20 mg/kg), showed a significant inhibition of tumor growth. In the small tumor model (50 mm^3), the lifespan of 4T1 tumor-bearing mice treated with ^{188}Re-liposomes and Lipo-DOX was increased by 21.7 and 169.6%, respectively, compared to those treated with normal saline. In the large tumor model (300 mm^3), the lifespan of the ^{188}Re-liposomes and the Lipo-DOX treated group was also increased by 35.2 and 141.2%, respectively. In this study, it was found that Lipo-DOX is better than ^{188}Re-liposomes, for the treatment of 4T1 breast cancer. A further investigation of combined therapy, in a breast cancer model, using ^{188}Re-liposomes and Lipo-Dox, to determine whether a synergistic effect exists, is ongoing in our laboratory.

Correspondence to: Dr Te-Wei Lee, Isotope Application Division, Institute of Nuclear Energy Research, No. 1000 Wenhua Road, Chiaan Village, Lungtan, Taoyuan 325, Taiwan, R.O.C.
E-mail: twlee@iner.gov.tw

Key words: liposomal doxorubicin, ^{188}Re-liposomes, therapeutic efficacy, 4T1 murine breast tumor

Introduction

To improve the pharmacokinetic properties and to reduce the toxicity of anticancer drugs, liposome has been considered as a promising carrier for cancer therapeutics (1,2). Since liposome accumulates passively, at the target tumor site, via the enhanced permeability and retention (EPR) effect (2), the prolonged intratumoral drug retention provides a drug with a better chance of killing tumor cells (3). Liposomal drugs, such as pegylated liposomal doxorubicin (Lipo-DOX), have been shown to increase antitumor activity, in a variety of preclinical and clinical studies (4-6). In addition, a reduced toxicity of Lipo-DOX has been reported, when compared to that of free DOX (7,8). Due to the enhanced accumulation and increased therapeutic efficacy, Lipo-Dox is currently approved for use in refractory ovarian cancer and metastatic breast cancer (9,10).

There has been considerable recent interest in the development of new radiopharmaceuticals, suitable for radiotherapy and diagnostic imaging. Preclinical studies of tumor therapy with liposome-mediated radiopharmaceuticals have been reported (11,12). Several beta-emitting radioisotopes, such as Iodine-131 (^{131}I), Yttrium-90 (^{90}Y), Rhenium-186 (^{186}Re) and Lutetium-177 (^{177}Lu), have been employed to treat cancer lesions (13-15). Rhenium-188 (^{188}Re) is an ideal radionuclide for therapeutic use, due to its maximum beta emission of 2.12 MeV, with a short physical half-life of 16.9 h and its 155 keV γ emission, for imaging purposes (16). The short physical half-life of ^{188}Re allows for higher activity than other radionuclides with long half-life. In addition, ^{188}Re can be obtained from a ^{188}W/^{188}Re generator, which makes it convenient for research and routine clinical uses.

Our previous findings suggest that ^{188}Re-N,N-bis (2-mercaptoethyl)-N',N'-diethylethylenediamine (BMEDA)-

labeled pegylated liposomes (^{188}Re-liposomes) is a promising agent for cancer therapy, in C26 murine colon carcinoma ascites (17,18), the solid tumor model (19,20) and the human colorectal solid tumor model (21). To evaluate the therapeutic potential of ^{188}Re-liposomes in other tumor types, the poorly immunogenic BALB/c mouse-derived 4T1 mammary carcinoma (22,23), which shares many characteristics with its human counterpart mammary carcinoma, was used to model human breast cancer. The 4T1 tumor is highly tumorigenic and can spontaneously metastasize, from the primary tumor in the mammary gland, to multiple distant sites, including lymph nodes, blood, liver, lung, brain and bone (24,25). The 4T1 orthotopic breast cancer model has been validated, in drug efficacy and toxicity studies (26-28); therefore, this model is appropriate for the assessment of the therapeutic efficacy of new pharmaceuticals.

Since there is little information concerning a comparison of antitumor activity, between radiopharmaceuticals and Lipo-DOX, this study proposes to investigate the therapeutic efficacy of ^{188}Re-liposomes and Lipo-DOX, on the 4T1 murine orthotopic breast cancer model, using a single intravenous injection. Both a bio-distribution assay and SPECT/CT imaging were performed, to determine the uptake of ^{188}Re-liposomes in 4T1 tumors. Significant differences in the inhibition of tumor growth were observed between the group treated with ^{188}Re-liposomes (4/5 MTD, 29.6 MBq), Lipo-DOX (4/5 MTD, 20 mg/kg) and that treated with normal saline. According to the results for survival rate and increased lifespan, Lipo-DOX is better than ^{188}Re-liposomes for the treatment of 4T1 murine orthotopic breast cancer.

Materials and methods

Materials. The pegylated liposome was purchased from Taiwan Liposome Co. (Taipei, Taiwan), with an average particle size about 82.59 nm. The lipid compositions of the liposome contain hydrogen soybean phosphatidylchloine (HSPC), cholesterol, polyethylene glycol-(1,2-distearoyl-sn-glycero-3-phosphatidylethanolamine) (PEG-DSPE) (molar ratio 3:2:0.3) and ammonium sulfate solution, with 250 mM [$(NH_4)_2SO_4$, pH 5.0], in inner water phase. The final concentration of liposomes was estimated by phosphate assay (29). It was found to contain 13.16 μmol/ml phospholipids.

The ^{188}W/^{188}Re generator was purchased from Oak Ridge National Laboratory (Oak Ridge, USA). Elution of the ^{188}W/^{188}Re generator with normal saline provided solutions of carrier-free ^{188}Re, as sodium perrhenate ($NaReO_4$). N,N-bis (2-mercaptoethyl)-N',N'-diethylethylenediamine (BMEDA) was purchased from ABX (Radeberg, Germany). The PD-10 column was purchased from GE Healthcare (Uppsala, Sweden). Cell culture materials were obtained from Gibco-BRL (Grand Island, NY, USA). All other chemicals were purchased from Merck (Darmstad, Germany).

Cell line and orthotopic breast cancer model. The 4T1 murine breast cancer cell line (kindly supplied by Dr Keng-Li Lan) was grown in α-MEM medium, supplemented with 10% fetal bovine serum (FBS), at 37˚C, in 5% CO_2. Cells were detached with 0.05% trypsin/0.53 mM EDTA, in Hanks' Balanced Salt Solution (HBSS). Five-week-old female BALB/c mice were purchased from the National Animal Center of Taiwan (Taipei, Taiwan), with food and water being provided, *ad libitum*, in the animal house of the Institute of Nuclear Energy Research (INER), Taoyuan (Taiwan). Animal protocols were approved by the Institutional Animal Care and Use Committee (IACUC) at the INER. Mice were inoculated with $2x10^5$ 4T1 tumor cells, in the mammary fat pad, and the tumors were allowed to grow, until they reached the particular size necessary for each experiment.

Preparation of ^{188}Re-liposomes. The labeling method for BMEDA radiolabeled with ^{188}Re was as previously described (18,20,30). The labeling efficiency of ^{188}Re-BMEDA complex was determined using ITLC-SG paper chromatography, eluted in normal saline. The process moved to the next step, when the labeling efficiency of ^{188}Re-BMEDA complex had reached >98%.

The preparation of ^{188}Re-liposomes proceeded as follows. Briefly, pegylated liposomes (1 ml, 13.16 μmol phospholipids) were added to the ^{188}Re-BMEDA solution and incubated, at 60˚C, for 30 min. The ^{188}Re-liposomes were separated from free ^{188}Re-BMEDA and purified, using a PD-10 column, eluted with normal saline. In this study, the labeling efficiency of ^{188}Re-liposomes was >80% (determined by the activity in pegylated liposomes, after separation, divided by the total activity, before separation).

Bio-distribution studies. Twenty mice (5 mice per each time-point) were intravenously injected with 1.3 MBq/100 μl of ^{188}Re-liposomes (phospholipid 0.09 μmol), after their tumors reached ~300 mm^3 in size. At different time-points (1, 4, 24 and 48 h), the mice were sacrificed, by CO_2 asphyxiation. Blood samples were collected, via cardiac puncture. Organs of interest were removed, washed and weighed and the radioactivity was measured with a Cobra II Auto-Gamma Counter (Packard). The results were expressed as the percentage injected dose per gram of tissue (%ID/g).

Planar and micro-SPECT/CT imaging. Both planar and SPECT/CT imaging were performed, using a micro-SPECT/CT scanner (X-SPECT/CT, Gamma Medica, Northridge, CA), with low-energy, high-resolution collimators. Planar images of the anesthetized mice (n=2) with 300 mm^3 4T1 breast tumors were collected at 1, 4 and 24 h after intravenous injection of 11.1 MBq/100 μl ^{188}Re-liposomes (0.58 μmol phospholipids). Acquisition time was 10 min per image. The planar images acquired at each time-point were analyzed, to determine the %ID/g in the tumor. A region of interest (ROI) was drawn around the standard source, to obtain counts for the activity (mCi) conversion factor. To determine the %ID/g in the tumor, a ROI was drawn over the tumor, in the lateral images, and the counts obtained were converted to activity values.

The micro-SPECT/CT images, with the animal in exactly the same position, were obtained immediately after planar imaging. The procedure for micro-SPECT/CT imaging has been previously described (17). The imaging was accomplished using 64 projections, at 60 sec per projection. SPECT imaging was followed by CT image acquisition (X-ray source: 50 kVp, 0.4 mA; 256 projections). The software provided with X-SPECT/CT was used for the SPECT and CT image reconstruction, including the SPECT/CT image fusion. The SPECT images were reconstructed, to produce image sizes

Table I. Bio-distributions of ^{188}Re-liposomes, in 4T1 tumor-bearing mice, at 1, 4, 24 and 48 h after intravenous injection of 1.3 MBq/100 μl of ^{188}Re-liposomes.

Organ	1 h	4 h	24 h	48 h
Whole blood	22.54±2.12	18.10±0.88	4.77±0.70	1.13±0.15
Brain	0.46±0.11	0.37±0.05	0.11±0.01	0.04±0.01
Skin	0.81±0.49	0.76±0.21	0.69±0.30	0.41±0.09
Muscle	0.37±0.06	0.57±0.27	0.26±0.09	0.12±0.03
Bone	0.93±0.19	0.78±0.23	0.39±0.09	0.18±0.08
Pancreas	1.03±0.12	0.87±0.10	0.30±0.12	0.12±0.06
Spleen	3.18±0.46	4.25±0.40	3.04±1.07	1.45±0.30
Kidney	5.03±0.79	4.92±0.25	2.24±0.31	0.97±0.07
Liver	6.82±0.47	6.93±0.16	4.55±0.55	1.86±0.07
Heart	2.29±0.31	2.32±0.43	0.78±0.08	0.36±0.04
Lung	4.78±1.09	4.26±0.76	1.26±0.24	0.48±0.02
Stomach	3.03±0.70	1.37±0.34	0.96±0.14	0.33±0.03
Small intestine	2.84±1.04	3.35±1.26	2.54±1.12	0.83±0.10
Large intestine	0.73±0.13	0.99±0.16	0.92±0.27	0.36±0.08
Bladder	0.97±0.05	0.72±0.12	0.38±0.08	0.15±0.03
4T1 tumor	1.31±0.10	2.70±0.50	3.03±0.29	1.97±0.37
Feces	0.24±0.01	2.96±1.02	4.70±1.79	2.07±0.68
Tumor/Muscle	3.60±0.40	4.62±1.91	12.35±6.64	16.81±4.70

Data are expressed as percentages of injected dose per gram (%ID/g ± SD, n=5, at each indicated time-point).

of 56x56x56 (pixels), with an image resolution of 1.2 mm. The CT images were also reconstructed, in image sizes of 512x512x512 (pixels), with 0.15 mm image resolution.

Maximum tolerated dose. The toxicity of ^{188}Re-liposomes and Lipo-DOX were determined by a maximum tolerated dose (MTD) study. Forty female BALB/c mice, with an average age of 8 weeks and average weight of 20.6 g, were randomly divided into groups of 5 mice. The mice were given 29.6, 37 and 44.4 MBq of ^{188}Re-liposomes (0.76 μmol phospholipids), or 16, 20, 25 and 30 mg/kg of Lipo-DOX in 300 μl, by single intravenous injection, respectively. The control group was administered with normal saline. Mice were weighed, twice per week and the survival rate of the mice was recorded, every day. The drug-induced toxicities (lethality and body weight loss) were monitored, during a 4-week period. The MTD was defined as the active dose below the dose resulting in either the death of any animal or a loss of body weight of >20% (31-33).

Therapeutic efficacy studies. Sixty female BALB/c mice were used. Each was inoculated with 2x10^5 4T1 tumor cells, in the mammary fat pad. In order to evaluate the relationship between the therapeutic efficacy and tumor size, both small and large tumor models were developed (30 mice per model). The sizes of 4T1 tumors, for the small and large tumor models, were 50 and 300 mm^3, respectively.

Once tumors were inoculated to the indicated size, the 4T1 tumor-bearing mice were divided randomly into three groups (10 mice per group) and then administered with ^{188}Re-liposomes (4/5 MTD, 29.6 MBq), Lipo-DOX (4/5 MTD, 20 mg/kg) or normal saline (control group), via tail vein injection. The size of tumor was measured, twice weekly, using calipers, to evaluate tumor growth. Tumor measurements were converted into tumor volumes (V), using the formula: $V = (YxW^2)/2$, where Y and W are the largest and smallest perpendicular diameters, respectively. All data are expressed as mean ± standard deviation. The mean tumor growth inhibition rate (MGI) was calculated according to the volume of the tumor, at day 14, after treatment. The survival ratio of mice was monitored every day, during a 50-day period.

Statistical analysis. All results were expressed as mean ± standard deviation. An unpaired t-test was performed, to compare the results from different groups. Differences were considered significant at a 95% confidence level ($P<0.05$).

Results

Bio-distribution study of ^{188}Re-liposomes. Bio-distributions of ^{188}Re-liposomes, in 4T1 tumor-bearing mice, at 1, 4, 24 and 48 h after intravenous injection, are listed in Table I. The highest uptake of ^{188}Re-liposomes in a 4T1 tumor was 3.03±0.29 (%ID/g), at 24 h after administration. The tumor to muscle (Tu/Mu) ratio of ^{188}Re-liposomes reached 4.62±1.91 and 12.35±6.64%, at 4 and 24 h after injection, respectively. The highest Tu/Mu ratio of ^{188}Re-liposomes uptake was 16.81±4.70%, at 48 h. In addition, >1% ^{188}Re-liposomes accumulated in the liver, spleen and 4T1 tumor, 48 h post-injection, but low levels of ^{188}Re-liposomes were found in the organs of the central nervous and musculoskeletal systems.

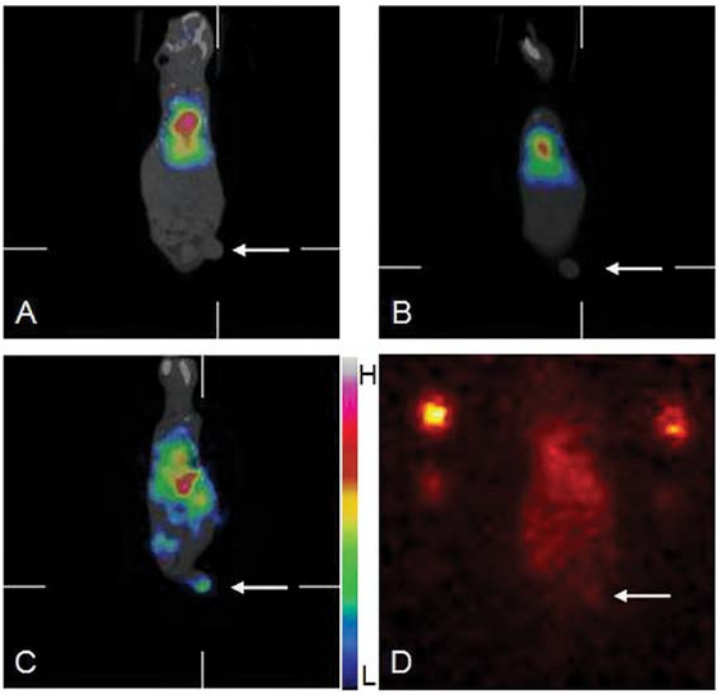

Figure 1. Micro-SPECT/CT and planar images of ^{188}Re-liposomes targeting 4T1 tumor-bearing mice. The ^{188}Re-liposomes containing 11.1 MBq of ^{188}Re were administered to each mouse by intravenous injection. The tomography images were acquired at (A) 1, (B) 4 and (C) 24 h, after injection. (D) The planar image was captured before tomography image acquisition, with the animal in the same position, at 24-h post-injection of ^{188}Re-liposomes. The arrows indicate the localization of 4T1 murine breast tumors in mice.

Micro-SPECT/CT and planar imaging analysis. To confirm the site-specific targeting of ^{188}Re-liposomes, both micro-SPECT/CT and planar imaging were performed, at 1, 4 and 24 h after intravenous injection. The micro-SPECT/CT imaging showed that a significant accumulation of ^{188}Re-liposomes was observed, in the 4T1 tumor, at 24 h (Fig. 1A-C). A high uptake of ^{188}Re-liposomes was also noted in the reticulo-endothelial system (RES) of the liver and spleen. The trend for tumor uptake, analyzed by micro-SPECT/CT imaging, is similar to the results of the bio-distribution study (Table I). A standard source of ^{188}Re-liposomes (0.07-1.11 MBq) was placed in the field of view, during planar image acquisition, for image quantification (Fig. 1D). According to the standard curve, the uptake of ^{188}Re-liposomes in the 4T1 tumor was ~4.59 %ID/g, at 24 h after administration. The value for uptake of ^{188}Re-liposomes was higher in the image quantification than in the bio-distribution study.

Maximum tolerated dose. To ascertain the MTD of ^{188}Re-liposomes and Lipo-DOX, female BALB/c mice with no 4T1 breast tumors were treated with various therapeutic doses. There was no significant decrease in body weight and drug-induced death, for normal mice administered with 29.6 and 37 MBq of ^{188}Re-liposomes. Similarly, there were no drug-induced toxicities (lethality and body weight loss) in normal mice, after administering 16, 20 and 25 mg/kg of Lipo-DOX. Therefore, the MTD of ^{188}Re-liposomes and Lipo-DOX were determined to be 37 MBq and 25 mg/kg, respectively.

Tumor growth inhibition and survival rate. Since our preliminary study found that colon carcinoma (C26)-bearing mice, injected with 4/5 MTD of ^{188}Re-liposomes, showed a significant inhibition of tumor growth and prolongation of lifespan (data not shown), a dose of 4/5 MTD was chosen, for this study of therapeutic efficacy. To evaluate the effect of tumor size on the therapeutic efficacy of ^{188}Re-liposomes and Lipo-DOX, both small and large tumor models were developed. The sizes of the 4T1 tumors for the small and large tumor models were 50 and 300 mm^3, respectively. After the tumors were inoculated to the desired size, the 4T1 tumor-bearing mice were administered with ^{188}Re-liposomes (4/5 MTD, 29.6 MBq), Lipo-DOX (4/5 MTD, 20 mg/kg) or normal saline (control group), via tail vein injection.

In the small tumor model, the tumor volumes for ^{188}Re-liposomes and control groups were 546±128 and

Table II. Therapeutic efficacy of Lipo-DOX and ^{188}Re-liposomes, in 4T1 tumor-bearing mice (small tumor model, 50 mm^3).

Treatment	MGI[a]	Median survival time (days)	P-value[b]	Lifespan[c] (%)
Lipo-DOX	0	59	<0.05	+169.6
^{188}Re-liposomes	0.49	28	<0.05	+21.7
Normal saline		23		

[a]MGI, mean growth inhibition rate = tumor volume of treated group/tumor volume of control group. [b]P-value was estimated by log-rank test, P<0.05 indicates significance. [c]Percentage of increased lifespan is expressed as (T/C-1) x100%, where T is the median survival time of a treated group and C is the median survival time of the control group.

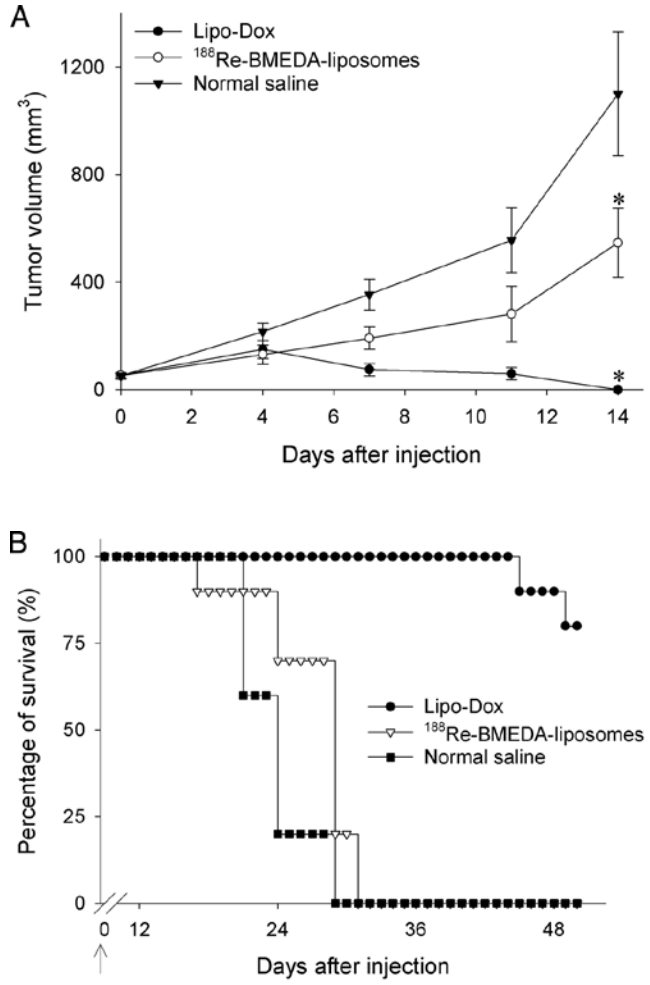

Figure 2. Tumor growth and survival curve for the 4T1 tumor-bearing mice (tumor size, ~50 mm^3). (A) Tumor volume (mm^3) vs. time (days), for female BALB/c mice implanted with 4T1 murine breast tumors, after administering ^{188}Re-liposomes (4/5 MTD, 29.6 MBq), Lipo-DOX (4/5 MTD, 20 mg/kg) or normal saline, by intravenous injection. (B) Survival rate for female BALB/c mice implanted with 4T1 murine breast tumors, after administering ^{188}Re-liposomes (4/5 MTD, 29.6 MBq), Lipo-Dox (4/5 MTD, 20 mg/kg) or normal saline, by intravenous injection. P-values, for the comparison of survival curves for various treatment groups, are listed in Table II.

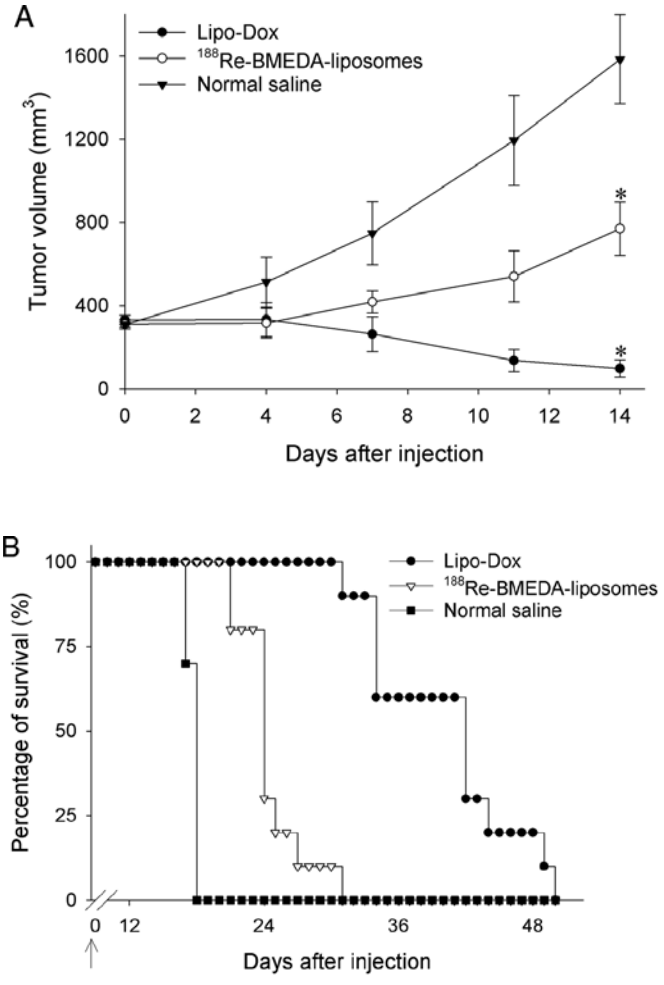

Figure 3. Tumor growth and survival curve for the 4T1 tumor-bearing mice (tumor size, ~300 mm^3). (A) Tumor volume (mm^3) vs. time (days), for female BALB/c mice implanted with 4T1 murine breast tumors, after administering ^{188}Re-liposomes (4/5 MTD, 29.6 MBq), Lipo-DOX (4/5 MTD, 20 mg/kg), or normal saline, by intravenous injection. (B) Survival rate for female BALB/c mice implanted with 4T1 murine breast tumors, after administering ^{188}Re-liposomes (4/5 MTD, 29.6 MBq), Lipo-DOX (4/5 MTD, 20 mg/kg), or normal saline, by intravenous injection. P-values, for comparison of the survival curves for various treatment groups, are listed in Table III.

1100±231 mm^3, respectively, at day 14. The tumors in the Lipo-DOX group disappeared at day 14. As shown in Fig. 2B, the survival rate for the Lipo-DOX group was 80%, at day 50, but the mice in the ^{188}Re-liposomes and control group were all dead, at day 31 and 29, respectively. The median survival time and lifespan of the 4T1 tumor-bearing mice in the small tumor model are detailed in Table II. The median survival times for the mice treated with ^{188}Re-liposomes, Lipo-DOX and normal saline were day 28 ($P<0.05$), day 59 ($P<0.05$) and day 23, respectively. Thus, the lifespan of the 4T1 tumor-bearing mice was increased in both the ^{188}Re-liposomes (+21.7%) and Lipo-DOX (+169.6%) treated groups.

In the large tumor model, the tumor volumes of the ^{188}Re-liposomes, Lipo-DOX and control groups were 771±128, 98±43 and 1584±213 mm^3, respectively, at day 14 (Fig. 3A). The survival rates for the various groups are also compared in Fig. 3B and these data are summarized in Table III. The mean growth inhibition rates, achieved by ^{188}Re-liposomes and Lipo-DOX, were 0.486 and 0.062, respectively. In the ^{188}Re-liposomes treated group, the lifespan of the 4T1 tumor-bearing mice was increased by 39.2% compared to the control group. In addition, the lifespan of 4T1 tumor-bearing mice administered with Lipo-DOX was increased by 141.2%. According to the data for this study of therapeutic efficacy, both ^{188}Re-liposomes and Lipo-DOX increased the lifespan of 4T1 tumor-bearing mice, but Lipo-DOX is better than ^{188}Re-liposomes, in both the small and the large tumor models.

Discussion

During the past decade, many studies have focused on developing animal models to test the efficacy of anticancer therapies. In our laboratory, several tumor models have been established, for preclinical testing of radiopharmaceuticals. These include prostate cancer, colon cancer and breast cancer. Since our previous findings suggest that ^{188}Re-liposomes is a

Table III. Therapeutic efficacy of Lipo-DOX and ^{188}Re-liposomes, in 4T1 tumor-bearing mice (large tumor model, 300 mm^3).

Treatment	MGI[a]	Median survival time (days)	P-value[b]	Lifespan[c] (%)
Lipo-DOX	0.062	41	<0.05	+141.2
^{188}Re-liposomes	0.486	23	<0.05	+35.2
Normal saline		17		

[a]MGI, mean growth inhibition rate = tumor volume of treated group/ tumor volume of control group. [b]P-value was estimated by log-rank test, P<0.05 indicates significance. [c]Percentage of increased lifespan is expressed as (T/C-1) x100%, where T is the median survival time of a treated group and C is the median survival time of the control group.

promising candidate for cancer therapy, in C26 murine colon carcinoma ascites (17,18), the solid tumor model (19,20) and the human colorectal solid tumor model (21), this study set out to investigate the therapeutic potential of ^{188}Re-liposomes, in breast cancer.

Breast cancer is a major cause of death, in women, and the median survival time, for patients with advanced metastasis, is approximately 18 months (34). Many chemotherapeutical agents, such as anthracyclines and taxanes, are commonly used as first-line therapy, for patients with metastatic breast cancer (35). Due to the reduced cytotoxicity, enhanced accumulation and increased therapeutic efficacy, Lipo-Dox is currently approved for use in refractory ovarian cancer and metastatic breast cancer (9,10). Although Lipo-Dox is considered to be a well-tolerated and effective option, as maintenance therapy, to delay tumor progression, no curative treatment is available for metastatic breast cancer (34,35). Therefore, new therapeutic strategies, such as the integration of chemotherapy and radiotherapy, are being explored (36,37).

In a therapeutic study of radiotherapy, several factors must be taken into consideration, including the animal model, the absorbed dose in the tumor and the radio-sensitivity of the tumor cells. In the bio-distribution study, similar results were obtained, for the uptake of ^{188}Re-liposomes by the tumor, for the 4T1 tumor (3.03±0.29 %ID/g) and C26 solid tumor (3.62±0.73 %ID/g), at 24 h post-injection (19). However, the therapeutic efficacy of ^{188}Re-liposomes, in the C26 solid tumor, was significantly better than that of ^{188}Re-liposomes in the 4T1 tumor, after administration with the same dose, indicating that the radio-sensitivity of tumor cells is a critical factor in radiotherapy.

It has long been recognized that tumor size is one of the indicators of therapeutic efficacy, for patients with metastatic cancers, especially in breast carcinoma (38). In this study, small (50 mm^3) and large (300 mm^3) tumor models were developed, to determine the effect of tumor size on radiotherapy. The lifespan of ^{188}Re-liposomes mice was increased in both the small (+21.7%) and large tumor models (39.2%), compared to the control (Tables II and III), but the therapeutic efficacy is better in the large tumor model than in the small tumor model. Although the precise relationship between tumor size and therapeutic efficacy is unclear, we propose that the tumor vasculature of the large tumor was suitable for the delivery and retention of ^{188}Re-liposomes.

A major focus of current research in radiotherapy is the optimization of schedules that combine radiation and molecular targeted therapies. Matsumura *et al* reported that radiotherapy improved the therapeutic efficacy of immunotherapy, by converting tumors into inflamed tissues (39,40). After exposure to ionizing radiation, enhanced production of pro-inflammatory chemokine CXCL16 was observed in 4T1 breast tumor cells, resulting in reduced tumor growth, *in vitro* and *in vivo*, by facilitating the effector phase of the antitumor immune response (39,40). In addition, Horton *et al* also demonstrated that Trastuzumab (Herceptin) enhanced the effectiveness of radiotherapy, by increasing radio-sensitivity, in breast cancer patients (36).

Since we are interested in discovering whether Lipo-DOX has a radio-sensitizing effect, in breast cancer, an investigation of combined therapy, in the breast cancer model, using ^{188}Re-liposomes and Lipo-Dox, is ongoing. Although our preliminary results show that pretreatment with Lipo-DOX significantly enhances the effect of ^{188}Re-liposomes, in the MDA-MB-231 breast tumor model (data not shown), further studies are needed, to determine the optimal use of radiotherapy, in combination with chemotherapy.

The tumor targeting and localization of ^{188}Re-liposomes, in the 4T1 tumors, was confirmed by the bio-distribution study and *in vivo* micro-SPECT/CT imaging. The highest uptake of ^{188}Re-liposomes, in a 4T1 tumor, was observed at 24 h after ^{188}Re-liposomes injection. According to the data for this study of therapeutic efficacy, both ^{188}Re-liposomes and Lipo-DOX significatly inhibit tumor growth and increase the lifespan of 4T1 tumor-bearing mice, in both the small and large tumor models. The present study is the first to evaluate the therapeutic efficacy of internal radiotherapy and chemotherapy, for treatment the of a 4T1 murine breast tumor. Although the results demonstrate that Lipo-Dox is better than ^{188}Re-liposomes, in the 4T1 murine orthotopic breast cancer model, the therapeutic potential of ^{188}Re-liposomes will be studied, in future investigations, by integration with Lipo-Dox, in the breast cancer model.

Acknowledgements

The authors would like to thank Chun-Lin Chen, for his help with the preparation of ^{188}Re and Cheng-Hui Chuang, for her technical assistance, in Micro-SPECT/CT imaging.

References

1. Allen TM and Cullis PR: Drug delivery systems: entering the mainstream. Science 303: 1818-1822, 2004.
2. Torchilin VP: Recent advances with liposomes as pharmaceutical carriers. Nat Rev Drug Discov 4: 145-160, 2005.
3. Gabizon A and Papahadjopoulos D: Liposome formulations with prolonged circulation time in blood and enhanced uptake by tumors. Proc Natl Acad Sci USA 85: 6949-6953, 1988.
4. Gabizon A, Catane R, Uziely B, *et al*: Prolonged circulation time and enhanced accumulation in malignant exudates of doxorubicin encapsulated in polyethylene-glycol coated liposomes. Cancer Res 54: 987-992, 1994.
5. Safra T, Muggia F, Jeffers S, *et al*: Pegylated liposomal doxorubicin (doxil): reduced clinical cardiotoxicity in patients reaching or exceeding cumulative doses of 500 mg/m^2. Ann Oncol 11: 1029-1033, 2000.

6. Marina NM, Cochrane D, Harney E, *et al*: Dose escalation and pharmacokinetics of pegylated liposomal doxorubicin (Doxil) in children with solid tumors: a pediatric oncology group study. Clin Cancer Res 8: 413-418, 2002.
7. Papahadjopoulos D, Allen TM, Gabizon A, *et al*: Sterically stabilized liposomes: improvements in pharmacokinetics and antitumor therapeutic efficacy. Proc Natl Acad Sci USA 88: 11460-11464, 1991.
8. Gabizon AA: Selective tumor localization and improved therapeutic index of anthracyclines encapsulated in long-circulating liposomes. Cancer Res 52: 891-896, 1992.
9. Gordon AN, Fleagle JT, Guthrie D, Parkin DE, Gore ME and Lacave AJ: Recurrent epithelial ovarian carcinoma: a randomized phase III study of pegylated liposomal doxorubicin versus topotecan. J Clin Oncol 19: 3312-3322, 2001.
10. O'Brien ME, Wigler N, Inbar M, *et al*: Reduced cardiotoxicity and comparable efficacy in a phase III trial of pegylated liposomal doxorubicin HCl (CAELYX/Doxil) versus conventional doxorubicin for first-line treatment of metastatic breast cancer. Ann Oncol 15: 440-449, 2004.
11. Emfietzoglou D, Kostarelos K, Papakostas A, *et al*: Liposome-mediated radiotherapeutics within avascular tumor spheroids: comparative dosimetry study for various radionuclides, liposome systems, and a targeting antibody. J Nucl Med 46: 89-97, 2005.
12. Emfietzoglou D, Kostarelos K and Sgouros G: An analytic dosimetry study for the use of radionuclide-liposome conjugates in internal radiotherapy. J Nucl Med 42: 499-504, 2001.
13. Witzig TE, Gordon LI, Cabanillas F, *et al*: Randomized controlled trial of yttrium-90-labeled ibritumomab tiuxetan radioimmunotherapy versus rituximab immunotherapy for patients with relapsed or refractory low-grade, follicular, or transformed B-cell non-Hodgkin's lymphoma. J Clin Oncol 20: 2453-2463, 2002.
14. Koppe MJ, Bleichrodt RP, Soede AC, *et al*: Biodistribution and therapeutic efficacy of $^{125/131}$I-, ^{186}Re-, $^{88/90}$Y-, or ^{177}Lu-labeled monoclonal antibody MN-14 to carcinoembryonic antigen in mice with small peritoneal metastases of colorectal origin. J Nucl Med 45: 1224-1232, 2004.
15. Lantry LE, Cappelletti E, Maddalena ME, *et al*: ^{177}Lu-AMBA: synthesis and characterization of a selective ^{177}Lu-labeled GRP-R agonist for systemic radiotherapy of prostate cancer. J Nucl Med 47: 1144-1152, 2006.
16. Iznaga-Escobar N: ^{188}Re-direct labeling of monoclonal antibodies for radioimmunotherapy of solid tumors: biodistribution, normal organ dosimetry, and toxicology. Nucl Med Biol 25: 441-447, 1998.
17. 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 34: 415-423, 2007.
18. Chen LC, Chang CH, Yu CY, *et al*: Pharmacokinetics, micro-SPECT/CT imaging and therapeutic efficacy of ^{188}Re-DXR-liposome in C26 colon carcinoma ascites mice model. Nucl Med Biol 35: 883-893, 2008.
19. 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 27: 2217-2225, 2007.
20. 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 37: 95-104, 2010.
21. Chen MH, Chang CH, Chang YJ, *et al*: MicroSPECT/CT imaging and pharmacokinetics of ^{188}Re-(DXR)-liposome in human colorectal adenocarcinoma-bearing mice. Anticancer Res 30: 65-72, 2010.
22. Aslakson CJ and Miller FR: Selective events in the metastatic process defined by analysis of the sequential dissemination of subpopulations of a mouse mammary tumor. Cancer Res 52: 1399-1405, 1992.
23. Miller FR, Miller BE and Heppner GH: Characterization of metastatic heterogeneity among subpopulations of a single mouse mammary tumor: heterogeneity in phenotypic stability. Invasion Metastasis 3: 22-31, 1983.
24. Pulaski BA and Ostrand-Rosenberg S: Reduction of established spontaneous mammary carcinoma metastases following immunotherapy with major histocompatibility complex class II and B7.1 cell-based tumor vaccines. Cancer Res 58: 1486-1493, 1998.
25. Lelekakis M, Moseley JM, Martin TJ, *et al*: A novel orthotopic model of breast cancer metastasis to bone. Clin Exp Metastasis 17: 163-170, 1999.
26. Moase EH, Qi W, Ishida T, *et al*: Anti-MUC-1 immunoliposomal doxorubicin in the treatment of murine models of metastatic breast cancer. Biochim Biophys Acta 1510: 43-55, 2001.
27. Laginha KM, Verwoert S, Charrois GJ and Allen TM: Determination of doxorubicin levels in whole tumor and tumor nuclei in murine breast cancer tumors. Clin Cancer Res 11: 6944-6949, 2005.
28. Wenzel J, Zeisig R and Fichtner I: Inhibition of metastasis in a murine 4T1 breast cancer model by liposomes preventing tumor cell-platelet interactions. Clin Exp Metastasis 27: 25-34, 2009.
29. Bartlett GR: Phosphorus assay in column chromatography. J Biol Chem 234: 466-468, 1959.
30. Bao A, Goins B, Klipper R, Negrete G and Phillips WT: ^{186}Re-liposome labeling using ^{186}Re-SNS/S complexes: in vitro stability, imaging, and biodistribution in rats. J Nucl Med 44: 1992-1999, 2003.
31. Cao S and Rustum YM: Synergistic antitumor activity of irinotecan in combination with 5-fluorouracil in rats bearing advanced colorectal cancer: role of drug sequence and dose. Cancer Res 60: 3717-3721, 2000.
32. Moore M, Hirte HW, Siu L, *et al*: Phase I study to determine the safety and pharmacokinetics of the novel Raf kinase and VEGFR inhibitor BAY 43-9006, administered for 28 days on/7 days off in patients with advanced, refractory solid tumors. Ann Oncol 16: 1688-1694, 2005.
33. Reilly RM, Chen P, Wang J, Scollard D, Cameron R and Vallis KA: 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 47: 1023-1031, 2006.
34. Bergh J, Jonsson PE, Glimelius B and Nygren P: A systematic overview of chemotherapy effects in breast cancer. Acta Oncol 40: 253-281, 2001.
35. O'Shaughnessy J: Extending survival with chemotherapy in metastatic breast cancer. Oncologist 10 (Suppl 3): S20-S29, 2005.
36. Horton JK, Halle J, Ferraro M, *et al*: Radiosensitization of chemotherapy-refractory, locally advanced or locally recurrent breast cancer with trastuzumab: a phase II trial. Int J Radiat Oncol Biol Phys 76: 998-1004, 2010.
37. Adamowicz K, Marczewska M and Jassem J: Combining systemic therapies with radiation in breast cancer. Cancer Treat Rev 35: 409-416, 2009.
38. Michaelson JS, Silverstein M, Wyatt J, *et al*: Predicting the survival of patients with breast carcinoma using tumor size. Cancer 95: 713-723, 2002.
39. Matsumura S and Demaria S: Up-regulation of the pro-inflammatory chemokine CXCL16 is a common response of tumor cells to ionizing radiation. Radiat Res 173: 418-425, 2010.
40. Matsumura S, Wang B, Kawashima N, *et al*: Radiation-induced CXCL16 release by breast cancer cells attracts effector T cells. J Immunol 181: 3099-3107, 2008.