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Interventional Therapy of Head and Neck Cancer with Lipid Nanoparticle-Carried Rhenium-186 Radionuclide

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Abstract

Purpose—Minimally invasive interventional cancer therapy of drug-carrying lipid nanoparticles (liposomes) via convection enhanced delivery generally applied by the use of an infusion pump can increase intratumoral drug concentration and retention while facilitating broad distribution throughout solid tumors. We investigated the utility of liposome-carrying β -emitting radionuclides to treat head and neck cancer in nude rats by direct intratumoral infusion.

Methods—Four groups of nude rats were subcutaneously inoculated with human tongue cancer cells. After tumors reached an average size of 1.6 cm³, the treatment group received an intratumoral infusion of liposomal rhenium-186 (¹⁸⁶Re) (185 MBq (5 mCi)/cm³ tumor). Three control groups were intratumorally infused with either, 1) unlabeled liposomes, 2) unencapsulated ¹⁸⁶Re-perrhenate, or 3) unencapsulated intermediate ¹⁸⁶Re-compound (¹⁸⁶Re-BMEDA). In vivo distribution of ¹⁸⁶Re-activity was measured by planar gamma camera imaging. Tumor therapy and toxicity were assessed by measurements of tumor size, body weight, and hematology.

Results—Average tumor volume of the ¹⁸⁶Re-liposome group on post-treatment day-14 decreased to 87.7±20.1%, while tumor volumes increased to 395.0% - 514.4% on average in other three groups ($P < 0.001$ vs ¹⁸⁶Re-liposome group). ¹⁸⁶Re-liposomes provided much higher intratumoral retention of ¹⁸⁶Re-activity, resulting in an average tumor radiation absorbed dose of 526.3±93.3 Gy, whereas ¹⁸⁶Re-perrhenate and ¹⁸⁶Re-BMEDA groups had only 3.3±1.2 and 13.4±9.2 Gy tumor doses respectively. No systemic toxicity was observed.

Conclusion—Liposomal ¹⁸⁶Re effectively treated the head and neck cancer with minimal side effects after convection enhanced interventional delivery. These results suggest the potential of liposomal ¹⁸⁶Re for clinical application in interventional therapy of cancer.

Introduction

Minimally invasive interventional drug delivery methods are emerging as viable treatment options for human cancer. These methods have been shown to extend survival, decrease

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normal organ toxicity, and improve quality of life in patients with solid tumors (1). The rationale of using this methodology for cancer therapy has been proven based on new developments in cancer biology research and recent clinical experiences with other minimally invasive therapies (2). Moreover, rapid advances in image guidance technologies have made accurate and effective application of locoregional therapy a clinically feasible option.

Among the therapeutic agents used for interventional cancer therapy, therapeutic radionuclides have advantages in their “straight-forward” mechanism of cancer cell eradication by radiation within their path range (3–5). The clinical use of yttrium-90 (^{90}Y)-microspheres for primary or metastatic liver lesions using image-guided intravascular delivery represents an example of this approach (1,6); however, intravascular delivery is restricted to lesions that allow the selective catheterization of tumor vasculature for the delivery of therapeutic agents.

Direct interstitial administration of therapeutic radiopharmaceuticals for solid cancer therapy has been investigated for many years (3). Commonly used β -emitting radionuclides have path lengths of a few millimeter that can practically treat a tumor, while delivering minimal radiation exposure to normal tissues (7,8). In treating larger tumors (> 1 cm), which are common in human cancers, the efficacy of tumor treatment among the patients will be further promoted by the following two strategies: 1) use of multiple injections delivered to different locations within the tumor (9); and 2) selection of therapeutic radiopharmaceuticals having the capability of dispersing effectively within the tumor (10).

Lipid nanoparticles (liposomes) have emerged as a potentially universal drug delivery system for chemo-, gene, and radionuclide therapies (11). To date, liposomes remain the most successful type of nanoparticles for drug delivery in disease treatment (12). A radiolabeling method to load $^{99\text{m}}\text{Tc}$, ^{186}Re and ^{188}Re into the interior aqueous space of post-manufactured liposomes with high efficiency, high stability and convenience, has been previously described (13–15). Previous distribution studies using $^{99\text{m}}\text{Tc}$ -labeled liposomes have shown that liposomes can achieve high sustained intratumoral concentrations after delivery by direct intratumoral injection into head and neck cancer, while also demonstrating a broad dispersion throughout the tumor (10).

Head and neck cancers are predominantly squamous cell carcinomas (SCC) that progress by local expansion and invasion of nearby structures, and locoregional lymph node metastasis (16), so that interventional drug delivery is a rational choice for these cancers (10). This paper reports our findings of head and neck cancer therapy using rhenium-186 (^{186}Re)-liposomes to treat large (1.6 cm³ on average) human head and neck SCC xenografts in nude rats by direct intratumoral convection-enhanced delivery (CED) (10,17). This delivery technique was chosen to allow the liposomes to disperse throughout the tumor interstitial space mediated by convection force (17).

Materials and Methods

Head and Neck SCC Xenografts in Nude Rats

Animal experiments were performed according to the NIH Animal Care and Use Guidelines and were approved by our Institutional Animal Care Committee. During each animal handling procedure, animals were anesthetized by inhalation of 1-3 % isoflurane (Vedco, Saint Joseph, MO) in 100 % oxygen using a veterinary inhalant anesthesia machine (Bickford, Wales Center, NY).

The head and neck SCC xenograft model used here has been previously reported (18). In brief, 5×10^6 SCC-4 cells (ATCC, Manassas, VA) in 0.2 ml serum free culture medium were inoculated to each male *rnu/rnu* athymic nude rat (Harlan, Indianapolis, IN) at 4 – 5 weeks age (75 – 100 g) on the dorsum at the level of the scapulae. SCC-4 is an established head and neck SCC cell line isolated from a human tongue cancer. Animals were examined daily by one of the authors to detect tumor growth. When the tumors became palpable, tumor size and animal weight was measured daily. Tumor size was obtained by measuring the length (*l*), width (*w*) and depth (*d*) of each tumor with calipers. Tumor volume was subsequently calculated by the ellipsoid volume formula, $V = (\pi/6)lwd$. When tumor volumes reached $\sim 1.5 \text{ cm}^3$ at day 14-15 after tumor cell inoculation, tumor therapy with ^{186}Re -liposomes was performed by convection-enhanced intratumoral administration (17).

To understand the role of liposome drug carrier for the interventional cancer therapy, three control groups with the intermediates in the preparation of ^{186}Re -liposomes: 1) unlabeled liposomes; 2) ^{186}Re -perrhenate; and 3) ^{186}Re -BMEDA were also included in the study.

Liposome Preparation

Liposomes of 100-nm diameter possessing the combination of ammonium sulfate pH and glutathione (GSH) gradients were prepared and characterized by a modified protocol described previously (19). The liposomes were comprised of distearoylphosphatidylcholine (DSPC) (Avanti Polar Lipids, Alabaster, AL), cholesterol (Calbiochem, San Diego, CA), and vitamin E (Sigma, St Louis, MO) (molar ratio 54:44:2). Lipid mixture was dissolved with chloroform, and then dried by rotary evaporation and overnight desiccation to form a lipid film. The dried lipid film was rehydrated with 300 mM sucrose in sterile water at 120 mM total lipid concentration and warmed to 55 °C followed by an overnight lyophilization. The dried lipid-sucrose mixture was rehydrated with 200 mM GSH (Sigma) and 300 mM ammonium sulfate (Sigma), pH 5.1, in sterile water at 60 mM total lipid concentration, then subjected to 5 freeze-thaw cycles, followed by sequential extrusion through polycarbonate filters with different pore sizes (2 μm , 1 μm , 400 nm, and 200 nm, 2 passes each; and 100 nm, 5 passes) at 55°C (Lipex Extruder, Northern Lipids, Vancouver, Canada). After extrusion, liposomes were repeatedly ultracentrifuged at 41,000 rpm (Ti 50.2 rotor, Beckman, Fullerton, CA) for 50 min and washed with 300 mM ammonium sulfate containing 75 mM sucrose, pH 5.1, in sterile water twice to remove any unencapsulated GSH. Liposome pellets were resuspended in 300 mM ammonium sulfate, pH 5.1 containing 300 mM sucrose in sterile water and stored at 4°C until needed.

Following manufacture, the particle size of the liposomes was measured with a 488-nm DLS laser light scattering instrument equipped with a DynaPro Dynamic Light Scattering system (Wyatt Technology, Santa Barbara, CA) and found to be $135.1 \pm 18.0 \text{ nm}$ in diameter. Phospholipid content was measured by the Stewart assay (20). Total lipid concentration of the liposomes was 40 mM, which was obtained from extrapolation from the measured phospholipid concentration and the proposed molar ratio for each liposome formulation. Liposomes were also checked for endotoxin levels and bacterial growth. No growth of bacteria was detected during 14-day culture. Endotoxin levels assayed with limulus amebocyte lysate (LAL) Pyrotell (Associates of Cape Cod Inc., E. Falmouth, MA) were $< 12.5 \text{ EU/ml}$.

^{186}Re -Liposome Preparation

The available $^{99\text{m}}\text{Tc}$, ^{186}Re and ^{188}Re radioactivity are in the chemical form of $^{99\text{m}}\text{Tc}$ -pertechnetate, ^{186}Re -perrhenate and ^{188}Re -perrhenate, respectively. To enable the loading of these radionuclides into liposomes, the radionuclides need to be reduced from valency VII to valency V, followed by coordination with the organic chemical, N,N-bis(2-mercaptoethyl)-

N',N'-diethyl-ethylenediamine (BMEDA). $^{99m}\text{Tc}/^{186}\text{Re}/^{188}\text{Re}$ -BMEDA can accumulate in the interior space of liposomes containing glutathione (GSH) and / or possessing a pH gradient between liposome interior and the bulk solution outside of the lipid nanoparticles (13–15).

The preparation of ^{186}Re -liposomes followed a previously reported protocol with minor modification in radioactivity amount and lipid amount of liposomes (19). In brief, 1.0 ml of pH 5.0 saline solution containing 1.34 mg BMEDA, 22.3 mg glucoheptonate, and 1.6 mg stannous chloride was placed in a new vial, flushed with nitrogen gas and sealed. ^{186}Re -perrhenate solution (6.66 GBq (180 mCi); University of Missouri Research Reactor, Columbia, MO) was added to the BMEDA-glucoheptonate-stannous chloride solution and incubated at 80 °C for 1 h. After incubation, the ^{186}Re -BMEDA solution was cooled to room temperature and adjusted to pH 7.0. A portion of the prepared ^{186}Re -BMEDA (2.22 GBq (60 mCi)) was set aside at this point for later injection in the ^{186}Re -BMEDA control group. Immediately before radiolabeling, 2.0 ml of liposomes (40 mM total lipid concentration) were eluted with PBS, pH 7.4 through a PD-10 column (GE Health Care, Uppsala, Sweden) to remove free ammonium sulfate and create GSH-ammonium pH gradients. Eluted liposomes were added to the ^{186}Re -BMEDA solution containing 4.44 GBq (120 mCi) ^{186}Re -activity and incubated for 1 h at 37 °C. Labeled ^{186}Re -liposomes were then separated from any free ^{186}Re using PD-10 columns and eluting with PBS, pH 7.4. The labeling efficiency of ^{186}Re -liposomes (% of ^{186}Re radioactivity associated with liposomes) was 75.9 %.

Intratumoral Drug Infusion and Image Acquisition

To understand the role of liposome drug carrier for the interventional cancer therapy, the tumor-bearing nude rats (23 in total) were divided into 4 groups with the experimental therapy group treated with ^{186}Re -liposomes, and the remaining 3 control groups injected with the intermediates in the preparation of ^{186}Re -liposomes by using the same infusion protocol: 1) unlabeled liposomes in the same lipid amount as ^{186}Re -liposomes; 2) ^{186}Re -perrhenate in the same amount of radioactivity as ^{186}Re -liposomes; and 3) ^{186}Re -BMEDA in the same amount of radioactivity as ^{186}Re -liposomes. The ^{186}Re activity for tumor treatment in Groups 2–4 was 185 MBq/cm³ tumor (5 mCi/cm³ tumor). To achieve the desired ^{186}Re activity delivery at the total infusion volume of 54 % of tumor volume (18 % each time, 3 times in total), ^{186}Re -perrhenate (Group 2), ^{186}Re -BMEDA (Group 3), and ^{186}Re -liposomes (Group 4) for animal study were diluted to 342.6 MBq/ml (9.26 mCi/ml) with PBS, pH 7.4. The total infused lipid amount of liposomes in Groups 1 and 4 was 6.17 $\mu\text{mole}/\text{cm}^3$ tumor (3.74 mg/cm³ tumor) (22.9 ± 7.1 and 25.1 ± 6.1 mg total lipid/kg body weight in Groups 1 and 4 respectively).

All animals were treated 14 days after tumor cell inoculation. The tumors in all groups had approximately the same average tumor volumes and tumor volume ranges. For all groups, tumors were infused using an infusion pump (KD Scientific, Holliston, MA) to deliver the assigned therapy volume and radioactivity. The rate of infusion was 0.5 ml/minute. A 23 gauge needle attached to an infusion pump by polyethylene tubing was inserted into the tumor as guided by direct visual placement and palpation. The total infusion volume for each tumor was 54 % of the tumor volume from caliper measurements determined on the day of the study. Each animal received a series of three percutaneous injections (18 % of total tumor volume with each injection) with 15-minute gap between each consecutive injection. The needle placements for each injection were equally spaced along the largest central section area of the tumor with each needle tip at ~1/3 depth in the tumor along the needle insertion direction.

Static planar images were acquired using a gamma camera (Picker International SX-300) equipped with a high resolution low-energy parallel hole collimator with a 40 % energy window centered over a 137-keV photo peak. The image acquisition period was 1 min initially, 2 min at 2 and 4 h, and 5 min at the remaining time points. Imaging was performed on all animals receiving ^{186}Re -containing infusions at strict time intervals of 0, 2, 4, 20, 68, 92, 116 and 140 h after treatment infusion. Images were acquired only at time points when in vivo radioactivity was detectable above background levels. Based on this criterion the endpoint for image acquisition was 20 h for the ^{186}Re -perrhenate group and 44 h for the ^{186}Re -BMEDA group. The ^{186}Re -liposome group was imaged to 140 h post-infusion and showed persistently high in vivo radioactivity through this interval. A ^{186}Re standard (8.88 MBq (0.24 mCi) initially) was included in each planar image for later use in quantification.

Image Analysis and Radiation Absorbed Dose Calculation

Image analysis and radiation absorbed dose calculation were performed as in previous studies (10,19). The retention of ^{186}Re activity within the tumor area and the distribution of activity to major organs were determined using ASIPro™ software (Siemens Medical Solutions Inc., Knoxville, TN). The percent of injected activity in each region-of-interest (ROI) of each planar image was calculated from the injected activity and the activity of the ^{186}Re -standard. After decay-correction, the cumulative radioactivity in the ROI is represented by the area under this curve and can be used to calculate the average radiation absorbed dose in the tumor by the following equation assuming a negligible contribution by the penetrative gamma energy (21):

$$D(\text{Gy}) = 7.31(\text{Gy} \cdot \text{g}/(\text{mCi} \cdot \text{h})) \times \tilde{A}(\text{mCi} \cdot \text{h})/m(\text{g})$$

In the above equation, D is the radiation absorbed dose in Gy, $\tilde{A}$ is the cumulative radioactivity as described above in $\text{mCi} \cdot \text{h}$, and m is the weight of the tumor tissue in g as calculated from the caliper measurement on treatment day 0.

Tumor Therapy Measurement and Toxicity Monitoring

Animals were monitored for treatment effects on tumor size, body weight, blood counts and general well-being for 6 weeks, or until animals reached the maximum allowable tumor burden. Groups 1-3 were euthanized by cervical dislocation under deep isoflurane sedation on post-treatment day 14 due to excessive tumor burden while animals treated with ^{186}Re -liposomes were followed for the entire 6 weeks prior to euthanization. Tumor size was measured daily by caliper measurement of length, width and depth of the tumor as described above. Body weight was also measured daily. Blood (500 μL) was obtained by tail vein venipuncture on day 0 (pre-treatment infusion), 3, 7, 10, and 14 with all groups, and also on day 20, 30, and 40 with Group 4. Blood was collected into tubes containing acid-citrate-dextrose anticoagulant (Ram Scientific Inc, Yonkers, NY) and delivered to University Hospital Pathology Laboratory (San Antonio, TX) for automated complete blood counts.

Histopathology

Histological evaluation was performed on tissues obtained from studied animals immediately after euthanasia. Tumor, kidney, liver and spleen specimens were dissected and fixed by immersion in 10 % formalin, then paraffin embedded, cut into 5 μm sections and hematoxylin and eosin (H&E) stained. Slides were first digitized at low-magnification using a high-resolution laser scanner (1,200 dpi) then analyzed by light microscopy and photographed with a digital camera (Canon PowerShot S3 IS) at 4 $\times$ and 20 $\times$ magnifications.

Data Analysis

Data analyses and column bar graphs used Excel software (Microsoft, Seattle, WA). Statistics of the percent activity in tumors and other organs, and the line graphs, including curve fitting, and the preparation of line graphs were performed with Origin 7.5 software (Origin Lab, Northampton, MA). Values from multiple samples are reported as average $\pm$ standard deviation (SD). Comparison between groups used one-way ANOVA and $P < 0.05$ was taken as significant difference.

Results

Tumor Volumes in ^{186}Re -liposome Treatment Group and Control Groups

On the day of treatment infusion (average body weight: 238.1 ± 35.9 g) (Day 0), the average tumor volumes for Groups 1, 2, 3, and 4 were 1.50 ± 0.52 , 1.69 ± 0.43 , 1.79 ± 0.39 , and 1.72 ± 0.45 cm^3 , respectively. Figure 1A shows the average tumor volumes for each treatment group over the duration of the study. The average tumor volume of the ^{186}Re -liposome treatment group decreased to 87.7 ± 20.1 % of the pre-treatment volume by day 14 ($P < 0.001$ compared to control groups). The rate of tumor growth over this period was -0.01 ± 0.02 cm^3/day . The lack of tumor growth allowed observation of tumor volumes in this group through the entire 6 weeks of the planned study duration. On the final day of the study (day 43), tumors in the ^{186}Re -liposome treated group were 103.1 ± 73.7 % of their initial volume (treatment day 0). Figure 1B shows the change in tumor size for each individual animal of the ^{186}Re -liposome treated group. Of these animals, 3 continued to show persistent tumor regression (50.6 ± 8.4 %), one tumor showed a nearly stable volume (115.3 %), and two tumors enlarged slowly, but at a significantly slower rate than the control groups (225.5 ± 1.3 %) at the conclusion of the study (day 43).

There was no significant difference in the overall progression of tumor growth between control groups that received unlabeled liposomes, ^{186}Re -perrhenate, or ^{186}Re -BMEDA. These animals all showed rapid tumor growth at a rate of 0.45 ± 0.19 cm^3/day in the unlabeled liposome group, 0.39 ± 0.12 cm^3/day in the ^{186}Re -perrhenate group, and 0.37 ± 0.11 cm^3/day in the ^{186}Re -BMEDA group. An increasing rate of tumor growth was observed by a similar factor in each control group over the duration of the study. By day 14, the average tumor volume in the control groups increased from 1.6 cm^3 to 7.4 cm^3 . There was an increase in tumor volume of 514.4 ± 59.1 % over treatment days 0 to 14 for the unlabeled liposome group, 426.4 ± 83.0 % in the ^{186}Re -perrhenate group, and 395.0 ± 41.2 % in the ^{186}Re -BMEDA group. Control animals were euthanized on day 14 because of excessive tumor burden.

Radioactivity Retention and Radiation Absorbed Dose

The % injected activity reported in this paper had no decay-correction; however, radiation absorbed dose calculation included radiation decay factor. Table 1 shows the intratumoral retention of injected radioactivity for each treatment group at various time points after injection. The ^{186}Re -liposome treated group retained 48.3 ± 6.0 % of the total injected activity within the tumor region immediately after the third injection. The ^{186}Re -liposome group also had sustained retention of radioactivity, maintained 8.9 ± 2.3 % of the injected radioactivity in the tumor region after 140 h. In contrast, ^{186}Re -BMEDA and ^{186}Re -perrhenate were both cleared rapidly from the tumors. After the third injection, these groups retained only 23.3 ± 4.9 % and 14.9 ± 6.6 % of total injected activity respectively. By 2 h the ^{186}Re -BMEDA treated group retained only 2.3 ± 1.4 % of the total injected activity at the tumor site, and by 44 h only 0.6 ± 0.8 % remained. The ^{186}Re -perrhenate group showed even less retention of radioactivity with only 1.0 ± 0.8 % remaining by 2 h, and only marginal retention in the tumor by 20 h. Figure 2 shows the average percent radioactivity

retained in the tumor (Fig 2A), and the total body radioactivity (Fig 2B) for each of the three animal groups receiving ^{186}Re -activity. Exponential decay curve fitting of the locoregional retention in the tumor has shown that the locoregional half-clearance time ($t_{1/2}$) was 54.6 h for the ^{186}Re -liposomes, 0.52 h for ^{186}Re -BMEDA, and 0.49 h for ^{186}Re -perrhenate (Table 2).

The total body clearance of injected radioactivity closely paralleled tumor clearance in each treatment group (Fig 2). The ^{186}Re -liposome treated group maintained 90 % of radioactivity until nearly 20 h post-injection, and 26.7 ± 3.6 % at 140 h. The ^{186}Re -BMEDA and ^{186}Re -perrhenate groups retained only 23.1 ± 5.4 % and 19.6 ± 5.4 % by 20 h. ^{186}Re -BMEDA and ^{186}Re -perrhenate activity cleared through abdominal organs (Fig 3). The ^{186}Re -liposomes showed early and delayed accumulation in liver and kidneys respectively as also demonstrated in previous studies (9) with a maximum of 9.6 ± 2.3 % of the injected activity being present in the abdominal viscera (including liver, kidneys, spleen, stomach, intestines, etc) at 116 h post-injection. The distribution of extra-tumoral radioactivity in the ^{186}Re -BMEDA and ^{186}Re -perrhenate groups was remarkably similar, which may reflect the ^{186}Re re-oxidization of the ^{186}Re -BMEDA compound in vivo. These groups showed their highest levels of radioactivity in the stomach, with lesser levels also detectable in other abdominal organs, which is consistent with published data for ^{186}Re -perrhenate (22). The maximum activity measured in the sum of abdominal viscera in these groups was 21.8 ± 5.3 % and 22.9 ± 4.8 % respectively, with both occurring at 20 h post-injection.

The average radiation absorbed dose by tumors in the ^{186}Re -liposome group was 526.0 ± 93.3 Gy (Table 3), but only 13.4 ± 9.2 Gy and 3.3 ± 1.2 Gy for the ^{186}Re -BMEDA and ^{186}Re -perrhenate groups respectively, although each group did receive the same injected radioactivity. These low radiation absorbed doses by ^{186}Re -BMEDA and ^{186}Re -perrhenate were not effective in controlling tumor growth (Fig 1A).

Tumor Histopathology

Figures 4A, 4C, and 4E show the H&E stain images from ^{186}Re -liposome group and Figures 4B, 4D, and 4F are H&E stained images from the unlabeled liposome control group. H&E stained slides of control tumors show lobules of basophilic cancer cells often with a necrotic center. Tumors are partitioned into lobules by eosinophilic bands of stromal tissue and extracellular matrix. Features seen on the slides are consistent with a moderate to poorly differentiated non-keratinizing squamous cell carcinoma: atypical squamous cells, frequent and abnormal mitotic figures, metaplastic cartilage formation, and dystrophic calcification (Fig 4B, 4D, 4F). Animals from the ^{186}Re -BMEDA and ^{186}Re -perrhenate groups look the same as animals that received no radioactivity. In contrast, ^{186}Re -liposome treated tumors all showed large areas of dense eosinophilic connective tissue stroma with collapsed lobules, some of which contain necrotic tumor cells. Within these areas there do not appear to be any viable cancer cells or tumor regrowth. Adjacent to these areas, each tumor had a region containing viable tumor cells but with less distinct and poorly organized stromal architecture (Fig 4C, 4E). This is in sharp contrast to the control tumors where delicate bands of supporting stroma interdigitates between nests of tumor cells (Fig 4B, 4D, 4F). Tumors that showed the greatest post-treatment reduction in size by caliper measurement had the smallest areas of viable tumor on histological examination. There were no histologic differences in the liver, spleen or kidneys between control and therapy rats.

Body Weight

Body weight in the treatment and control groups was remarkably similar overall (data not shown). Groups 1-3 all showed steady increases in body weight throughout the duration of the study. The ^{186}Re -liposome treated group showed a mild weight loss of 8.5 ± 3.0 % from

post-treatment day 0 to day 5. After day 5, the treatment group rebounded to a weight comparable to the control groups and continued to gain weight through day 43.

Hematology

No significant hematological effects were seen that could be attributed to either ^{186}Re or liposome administration. Also, no animal showed signs of infection or bleeding tendencies during the study. White blood cell counts in the unlabeled liposome, ^{186}Re -liposome, and ^{186}Re -BMEDA groups were essentially the same both pre and post treatment. Platelet counts were the most variable of the examined blood indices. Comparison of each treatment group with the unlabeled liposome group did not suggest any effects to platelet counts given the large variations present within and across each group.

Discussion

Local ^{186}Re retention in the tumor was much higher when delivered by a liposome nanocarrier, resulting in a 150-fold greater tumor-absorbed radiation dose in the ^{186}Re -liposome group than in other ^{186}Re groups. This high dose resulted in drastically reduced tumor size and rate of tumor growth in ^{186}Re -liposome treated animals. A better intratumoral dispersion with ^{186}Re -liposomes may have also contributed to the effective tumor therapy (10). Observations of the ^{186}Re -liposome treated tumors dissected at day 43 showed the interstitial tissue was not as well organized with less discrete separation of tumor cells within lobules in comparison with the control tumors. This suggests the tumor architecture required for supporting tumor growth (23–25) has been disrupted by treatment. Treatment with ^{186}Re -liposomes was also very well tolerated and did not produce any observable systemic side effects or severe local reactions. These results clearly show the advantages of using lipid nanoparticle-carried β -emission radionuclides for interstitially administered interventional cancer therapy.

A significant advantage of the use of β -emitting radionuclides carried with liposomes is that there is no requirement for a release mechanism in order for the therapy to be effective due to the a few millimeter average path length of the β -radiation. This path length also compensates to some degree for any lack of homogeneity achieved within the tumor after CED. Among potential radionuclides that can be chosen for this tumor therapy application, ^{186}Re represents a prime candidate; it has 89.2 h half life and decays primarily by emission of non-penetrative β -particles as well as 10 % of 137 KeV γ -ray, optimal for non-invasive imaging of the in vivo distribution (21). The non-penetrative radiation energy of ^{186}Re , which has a path length of 1.8 mm, accounts for 94 % of total radiation energy (7,8,21). Based on these characteristics, the effect of radiation from ^{186}Re can be confined to a concise and predictable area which maximizes the treatment effect on cancer tissue while greatly sparing surrounding normal tissue. In the present study, intratumoral administration resulted in much lower systemic radiation doses and fewer systemic side effects monitored by blood cell counts were also observed. A similar low toxicity after intra-operative administration of ^{186}Re -liposomes has also been observed (19).

The feasibility of using directly injected therapeutic agents in human cancer treatment relies on our ability to accurately deliver the treatment into tumor tissue without violating critical anatomic structures that may be nearby or superficial to the target area. Although there is still much progress to be made in this area, our research suggests that future technology yet to be developed to precisely guide the delivery of β -emission radiotherapeutics for therapy coverage to the entire tumor is practical for human cancer treatment (1). By using various radiological imaging techniques, the retention and distribution of therapeutic agents in the tumor can be monitored in real time to ensure complete tumor coverage (10,17). In addition, re-treatment of locoregional tumor recurrence is also possible, since this localized

radiotherapy has the short millimeter path length and minimal toxicity to normal structures surrounding a solid tumor.

Overall, direct intratumoral drug delivery circumvents the major obstacles to systemic delivery and has been investigated for cancer therapy using radionuclide (9,26–28), chemo- [29], immuno- (30) and gene therapy agents (31). Solid tumors characteristically have disorganized hypo-vasculature and a lack of functional lymphatics (32,33), which results in poor fluid exchange between the tumor interstitial space and its exterior environment and a high interstitial pressure in solid tumors. This unique solid tumor physiology prevents efficient vasculature-based drug delivery; conversely, this tumor structure can be an advantage because it may prevent rapid drug clearance and promote local retention of therapeutics delivered by direct intratumoral injection. These effects can be further enhanced by utilizing particulate therapeutic agents (9,10,26–28).

A limitation of this preclinical cancer therapy study was that an image-guidance system was not used for the infusion of the therapeutic agents to tumors because the treatment delivery could be easily performed by visual access with palpation of the subcutaneous xenograft tumor. Use of this delivery method in human cancer therapy will require establishment of a well-formulated image-guided drug delivery protocol. This protocol may include the use of CT and MR imaging to obtain tumor dimension/volume information and to characterize tumor microenvironment and the surrounding critical structures. In addition, image-monitoring or pre-treatment planning to characterize intratumoral retention and distribution to ensure adequate tumor coverage for effective therapy may be necessary. The liposome-based therapeutic system used in this study can be combined or alternatively labeled with either ^{99m}Tc diagnostic radionuclide or gadolinium-MR contrast agents to enable pre-treatment imaging evaluation (10,17).

Conclusion

Our results show that intratumoral infusion of ^{186}Re as part of a liposome carrier can significantly suppress tumor growth in a rat xenograft model of human head and neck squamous cell carcinoma compared with other ^{186}Re -intermediate small molecule agents. These results suggest great potential for the clinical application of ^{186}Re -liposomes in the treatment of head and neck cancer, and other applicable solid cancers. The application of liposomal radiotherapeutic agents for locoregional treatment may be especially valuable in cases of advanced or recurrent disease where surgery or chemo-radiotherapy is no longer an option, and also in those patients whose co-morbidities preclude current first-line therapies.

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Abbreviations

CED	convection-enhanced delivery
SCC	squamous cell carcinoma
ROI	region-of-interest

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Figure 1.
Average tumor growth trend of all four groups (A) and tumor growth trend of each individual tumor in each animal in ^{186}Re -liposome group (B).

Figure 2A

Figure 2B

Figure 2.

Comparison of the average percent of injected radioactivity present in tumors (A) and overall total body activity (B) in each group over time. The % injected activity after each injection was compared with the total activity that had already been injected into each tumor, but does not include the radioactivity which has not been injected into each animal yet.

Figure 3.

Planar gamma camera images depicting the distribution of ¹⁸⁶Re-activity at various time points after treatment infusion for each study group. There was much higher retention and slower clearance of ¹⁸⁶Re-liposomes in the tumor region compared with ¹⁸⁶Re-BMEDA and ¹⁸⁶Re-perrhenate. (White Arrow-Tumor, Short Arrow-Standard, Grey Arrow-Stomach, Arrowhead-Kidney)

Figure 4. H&E stained sections from control animal (B, D, F) and from ^{186}Re -liposome treated animal (A, C, E) at 1 $\times$, 4 $\times$, and 20 $\times$ magnifications.

Table 1

Percent local retention of injected ^{186}Re -activity (no decay-correction) after intratumoral infusion.

Therapy group	Baseline	2 h	4 h	20 h	44 h	68 h	92 h	116 h	140 h
^{186}Re -Liposomes	48.3 $\pm$ 6.0	44.2 $\pm$ 10.8	42.4 $\pm$ 10.5	39.7 $\pm$ 11.7	28.3 $\pm$ 7.4	18.8 $\pm$ 4.4	15.5 $\pm$ 3.4	11.4 $\pm$ 2.6	8.9 $\pm$ 2.3
^{186}Re -BMEDA	23.3 $\pm$ 4.9	2.3 $\pm$ 1.4	1.7 $\pm$ 1.1	0.7 $\pm$ 0.6	0.6 $\pm$ 0.8				
^{186}Re -Perthenate	14.9 $\pm$ 6.6	1.0 $\pm$ 0.8	0.2 $\pm$ 1.2	0.1 $\pm$ 0.2					

Table 2

Local clearance of ^{186}Re -activity after intratumoral injection for each treatment group.

Treatment	Immediate clearance (%)	Locoregional Clearance Time $T_{1/2}$ (h)	R^2
^{186}Re -Liposomes	51.7±6.0	54.55	0.993
^{186}Re -BMEDA	76.7±4.9	0.52	0.905
^{186}Re -Perrhenate	85.1±6.6	0.49	0.935

Table 3

Tumor radiation absorbed dose (Gy), and percent tumor volumes on Days 14 and 43 compared with Day 0 of ^{186}Re 4iposome treated animals.

Rat	Absorbed Dose (Gy)	Day 0 Tumor Volume (g)	Day 14 Tumor Volume (%)	Day 43 Tumor Volume (%)
1	417.3	1.1	60.6	58.5
6	519.6	1.6	123.1	227.3
16	579.5	1.6	84.5	38.6
18	650.7	2.0	84.6	115.3
20	419.9	1.6	88.7	54.8
24	570.9	2.4	84.9	223.7
Average $\pm$ SD	526.3 $\pm$ 93.3	1.7 $\pm$ 0.4	87.7 $\pm$ 20.1	119.7 $\pm$ 86.0