Melanoma Therapy via Peptide-Targeted α-Radiation

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Abstract

Purpose: The therapeutic efficacy of a unique melanoma-targeting peptide conjugated with an in vivo generated α-particle-emitting radionuclide was evaluated in the B16/F1 mouse melanoma model. α-Radiation is densely ionizing, resulting in high concentrations of destructive radicals and irreparable DNA double-strand breaks. This high linear energy transfer overcomes radiation-resistant tumor cells and oxygen effects resulting in potentially high therapeutic indices in tumors such as melanoma.

Experimental Design: The melanoma targeting peptide, 1,4,7,10-tetraazacyclodecane-1,4,7,10-tetraacetic acid (DOTA)-Re(Arg11)CCMSH, was radiolabeled with 212Pb, the parent of 212Bi, which decays via α and β decay. Biodistribution and therapy studies were done in the B16/F1 melanoma-bearing C57 mouse flank tumor model.

Results: 212Pb[DOTA]-Re(Arg11)CCMSH exhibited rapid tumor uptake and extended retention coupled with rapid whole body disappearance. Radiation dose delivered to the tumor was estimated to be 61 cGy/μCi 212Pb administered. Treatment of melanoma-bearing mice with 50, 100, and 200 μCi of 212Pb[DOTA]-Re(Arg11)CCMSH extended their mean survival to 22, 28, and 49.8 days, respectively, compared with the 14.6-day mean survival of the placebo control group. Forty-five percent of the mice receiving 200 μCi doses survived the study disease-free.

Conclusions: Treatment of B16/F1 murine melanoma – bearing mice with 212Pb[DOTA]-Re(Arg11)CCMSH significantly decreased tumor growth rates resulting in extended mean survival times, and in many cases, complete remission of disease. 212Pb-DOTA-Re(Arg11)CCMSH seems to be a very promising radiopharmaceutical for targeted radionuclide therapy of melanoma.

The incidence and mortality rates associated with melanoma have increased by 3% to 7% in recent years (1, 2). Early melanoma tumor diagnosis and prompt surgical removal are a patient’s best hope for a cure. Unfortunately, metastatic melanoma is resistant to current chemotherapy and immunotherapy regimens. Survival times for patients with lymph node metastases average 12 to 15 months, whereas patients with liver and bone metastases average 3 to 4 months (1). Despite extensive research, there have been no major improvements in advanced melanoma treatment outcomes during the past 30 years. Targeted radionuclide therapy, namely α-particle therapy, is a potentially important alternative to conventional therapeutic regimens. In comparison with external beam radiation therapy and chemotherapy, targeted radionuclide therapy offers the potential of tumor-selective radiotherapeutic treatment of distal metastases whereas sparing normal tissues and organs.

α-Particles are doubly-charged helium ions with energies of 5 to 9 MeV characterized by high linear energy transfer over short path lengths (30-90 μm; refs. 3, 4). Only a few α-particle traversals per cell are necessary to cause irreversible damage resulting in cell death (5). Cytotoxicity of α-particle radiation is independent of dose rate (4) and unaffected by tissue oxygen levels (6), which allow tumors with hypoxic regions to be effectively irradiated. The short range and high ionization density associated with α-particles contribute to highly specific destruction within tumors, whereas minimizing collateral damage of healthy tissues. Promising preclinical α-particle radioimmunotherapy results with leukemia (7, 8) lung (9), ovarian (10), and prostate cancers (11) have been shown. In addition, early clinical trials have highlighted the potential of 213Bi and 211At labeled immunoconjugates for α-radiotherapy (12, 13).

Over the past several years, our laboratory has developed a novel class of metal-cyclized melanotropin peptide analogues for melanoma imaging and therapy that target the melanocortin-1 receptor, which is overexpressed on melanoma tumor cells (14–16). Incorporation of a rhenium metal atom into the structure of the cyclic melanotropin peptide resulted in resistance to chemical and proteolytic degradation in vivo, whereas retaining high bioactivities. In this study, we report the melanoma therapeutic effects of targeted α-particle...
radiotherapy using the 1,4,7,10-tetraazacyclododecan-1,4,7,10-tetraacetic acid (DOTA)–conjugated melanotropin analogue, DOTA-\text{Re(11)}\text{CCMSH} (17), radiolabeled with \text{212Pb}. High tumor to normal tissue uptake ratios of \text{212Pb}\text{DOTA}-\text{Re(11)}\text{CCMSH} coupled with rapid whole body disappearance of activity, resulted in large and selective radiation doses to the tumors. Treatment of melanoma-bearing mice with \text{212Pb}\text{DOTA}-\text{Re(11)}\text{CCMSH} yielded a dose-dependent reduction in tumor growth and total eradication of many tumors at higher activity levels. Despite being classified as a radiation-resistant neoplasm, melanoma was efficaciously treated by peptide-targeted \(\alpha\)-radiation in tumor-bearing mice, highlighting the clinical potential of targeted \(\alpha\)-radiotherapy for disseminated melanoma.

### Materials and Methods

**Synthesis of \text{212Pb-DOTA-\text{Re(11)}\text{CCMSH}.** DOTA-\text{Re(11)}\text{CCMSH}, was synthesized and purified by a method previously described (17). DOTA-\text{Re(11)}\text{CCMSH} was radiolabeled with \text{212Pb} obtained from a \text{224Ra-212Pb/212Bi} radioisotope generator (ref. 18; AlphaMed, Inc., Acton, MA). Preferential elution of \text{212Pb} was done by rinsing the generator column with 0.5 N HCl to remove the majority of \text{212Bi} and its daughters followed by a 2 N HCl rinse to elute the \text{212Pb} along with any remaining \text{211Bi}. Shortly following the time of elution, \text{212Pb} was in dynamic equilibrium with \text{212Bi} and its daughters. DOTA-\text{Re(11)}\text{CCMSH} was radiolabeled by combining 100 to 350 \(\mu\)L of the generator eluant, 85 \(\mu\)L of 5 mol/L NaOH, 250 \(\mu\)L of 0.5 mol/L \text{NH}_3\text{OAc (pH 5.4)}, and 25 \(\mu\)L of 1 mg/mL DOTA-\text{Re(11)}\text{CCMSH} into a reaction vial and incubated at 75°C for 40 minutes. 25 \(\mu\)L of \text{212Pb-DOTA-\text{Re(11)}\text{CCMSH}} was purified to a single species using HPLC with a C-18 reverse phase analytic column. Prior to the HPLC collection, 25 mg of \(\alpha\)-ascorbic acid was added into the collection vial to minimize the radioisolation of \text{212Pb-DOTA-\text{Re(11)}\text{CCMSH}}. The radioactivity of \text{212Pb-DOTA-\text{Re(11)}\text{CCMSH}} preparation was determined immediately after HPLC purification in a Capintec CRC-15R dose calibrator calibrated with a standardized \text{212Pb} sample obtained from Pacific Northwest National Laboratory (Richland, WA). Purified peptide solutions were purged with nitrogen gas for 20 minutes to remove the acetonitrile and adjusted to pH 5 with 0.9% NaCl.

**Animal studies.** Animal studies were conducted in compliance with Institutional Animal Care and Use Committee approval. Pharmacokinetic and therapy studies were done in C57 black mice bearing B16/F1 murine melanoma tumors. Melanocortin-1 receptors on B16/F1 murine melanoma and human melanoma have indistinguishable affinities for the radiouclide targeting peptide (16), suggesting that the results obtained in the B16 melanoma model will be translatable to human melanoma models and potential clinical use. Mice were inoculated s.c. with \(1 \times 10^6\) B16/F1 murine melanoma cells in the right flank for biodistribution studies. When the weight of tumors reached \(0.2 \text{ to } 0.16\) g to 4.40 \(\mu\)Ci of \text{212Pb-DOTA-\text{Re(11)}\text{CCMSH}} was injected into each mouse through the tail vein. Groups of four mice per each time point were used for the biodistribution studies. The mice were sacrificed at 5 and 30 minutes, and 1, 2, 4, 24, and 48 hours postinjection, and tumors and organs of interest were harvested, weighed and counted in a Wallac 1480 automated gamma counter using the following energy windows, \text{211Pb} (90 to 260 keV and \text{212Bi} (420 to 810 keV). Blood values were taken as 6.5% of the whole body weight. The results were expressed as a percentage of the injected dose per gram (%ID/g) and as the percentage of injected dose (%ID). The tumor uptake specificity of \text{212Pb-DOTA-\text{Re(11)}\text{CCMSH}} was determined by blocking tumor uptake at 2 hours postinjection with the coinjection of 10 \(\mu\)g of unlabeled NDP (19), a linear \(\alpha\)-MSH peptide analogue with picomolar affinity for the \(\alpha\)-MSH receptor present on murine melanoma cells.

### Results

DOTA-\text{Re(11)}\text{CCMSH} was radiolabeled with \text{212Pb} (Fig. 1) obtained from a \text{224Ra-212Pb/212Bi} radioisotope generator. Lead-212 (\(1/2 = 10.6\) hours) decays to \text{212Bi} (\(1/2 = 1.01\) hours) via \(\beta\)-emission that subsequently decays via a branched

**Dosimetry studies.** The biodistribution of \text{212Pb-DOTA-\text{Re(11)}\text{CCMSH}} over time was determined to calculate radiation absorbed doses from \text{212Pb-DOTA-\text{Re(11)}\text{CCMSH}} in tumor and normal organs and tissues using methods described previously (20–22). Time-activity curves were generated for 13 organs and tissues (blood, brain, heart, lung, liver, spleen, stomach, kidney, small intestine, muscle, pancreas, and tumor). Cumulative activities of \text{211Bi} and \text{212Bi} were determined for each organ by integrating the area under the time-activity curves. The cumulative activities were then used with a dosimetric model (21, 22) developed specifically for the laboratory mouse, accounting for the \(\alpha\)- and \(\beta\)-radiation deposited locally. The dosimetric model is used to evaluate dose from activity within tissues as well as the cross-organ \(\beta\) dose contributions.

**Radionuclide therapy of \text{212Pb-DOTA-\text{Re(11)}\text{CCMSH}.** The therapeutic efficacy of \text{212Pb-DOTA-\text{Re(11)}\text{CCMSH}} was examined in B16/F1 murine melanoma – bearing C57 mice. C57 mice were inoculated s.c. with \(1 \times 10^6\) B16/F1 murine melanoma cells in the right flank. Palpable dark melanoma tumors were observed 3 days following tumor cell inoculation. Three treatment groups of 8 to 10 mice were administrated single doses of 50, 100, and 200 \(\mu\)Ci of \text{212Pb-DOTA-\text{Re(11)}\text{CCMSH}} through the tail vein on the 4th day after tumor cell implantation. An untreated tumor control group received 100 \(\mu\)L of normal saline. After the administration of the therapeutic infusion, tumor size, body weight, and animal body condition were determined daily. Tumor volume was calculated by measuring the length, width, and depth of the tumors with a caliper and using the following formula: tumor volume = \((\text{length} \times \text{width} \times \text{depth}) \div \pi \div 6\). Mice were removed from the therapy study and sacrificed if body weight loss was >20% of initial body weight, tumor size exceeded 1.0 cm\(^3\), or the appearance of skin ulcerations at the tumor site. The total study period was 120 days, which was 10 times the average survival period of untreated tumor-bearing animals. Toxicity of \text{212Pb-DOTA-\text{Re(11)}\text{CCMSH}} to the kidneys of 100 and 200 \(\mu\)Ci treatment groups was evaluated by pathologic examination after completion of the therapy study. The kidney and tumor site skin biopsies from therapy animals were examined by a veterinary pathologist at the University of Missouri School of Veterinary Medicine Research Animal Diagnostic Laboratory. Kaplan-Meier survival curves were obtained by using SPSS software (SPSS Inc., Chicago, IL). Statistical analysis was done using Student’s \(t\) test for unpaired data. A 95% confidence level was chosen to determine the significance between untreated and treated groups, with \(P < 0.05\) being significantly different.

![Fig. 1. A schematic structure of the rhenium-cyclized peptide, \text{212Pb-DOTA-Re(11)}\text{CCMSH.}](image)
pathway to stable $^{208}$Pb, yielding an α-particle and several β and γ emissions (3, 4). The biodistribution and tumor-targeting properties of $^{212}$Pb-DOTA-Re(Arg$^{11}$)CCMSH were determined in B16/F1 murine melanoma–bearing C57 mice (Table 1). $^{212}$Pb-DOTA-Re(Arg$^{11}$)CCMSH exhibited rapid accumulation and high retention in the melanoma tumors. The tumor uptake values of $^{212}$Pb-DOTA-Re(Arg$^{11}$)CCMSH reached a maximum level of 13.49% injected dose per gram (%ID/g) at 5 minutes postinjection. Tumor activity levels remained constant over 4 hours, and then gradually declined to 4.59% ID/g at 24 hours postinjection. Receptor-mediated tumor uptake of $^{212}$Pb-DOTA-Re(Arg$^{11}$)CCMSH was very rapid, with ~90% of the administered radioactivity washed out of the body by 2 hours postinjection. Very little radioactivity remained in blood and major organs except for the kidneys, which was the primary route of excretion for $^{212}$Pb-DOTA-Re(Arg$^{11}$)CCMSH. Coinjection of the super potent melanotropin analogue [Nle 4, D-Phe 7]melanotropin against the tumor uptake was specific and receptor-mediated. Whole body disappearance of $^{212}$Pb-DOTA-Re(Arg$^{11}$)CCMSH was very rapid, with ~90% of the administered radioactivity washed out of the body by 2 hours postinjection. Very little radioactivity remained in blood and major organs at 2 hours postinjection, including the liver, lung, and muscle except for the kidneys, which was the primary route of excretion for $^{212}$Pb-DOTA-Re(Arg$^{11}$)CCMSH. Coinjection of NDP did not affect kidney uptake, and therefore the kidney activity was likely due to nonspecific peptide retention. Biodistribution results of the radiolabeled peptide, monitored in the $^{212}$Pb and $^{212}$Bi energy windows, was not significantly different with respect to radioactivity uptake and retention in the tumor and major organs including the kidneys (data not shown). The absorbed radiation doses to tumor and normal organs from $^{212}$Pb-DOTA-Re(Arg$^{11}$)CCMSH were estimated in this study based on the biodistribution data in B16/F1 murine melanoma–bearing mice (Table 2). Dosimetry calculations were based on energy deposition from the β-decay of $^{212}$Pb as well as the α, β, and γ-radiations of $^{212}$Bi (21, 22). The absorbed dose from $^{212}$Pb-DOTA-Re(Arg$^{11}$)CCMSH to the B16/F1 mouse tumor was 612 Gy/37 MBq (mCi). The high tumor dose was directly related to the rapid uptake kinetics and retention of the $^{212}$Pb-labeled peptide. Normal tissue doses were low except for the kidneys, which were estimated at 361 Gy/37 MBq. These results suggest that the kidneys will be the dose-limiting normal organ.

Therapy studies were done with C57 mice inoculated s.c. with $1 \times 10^6$ B16/F1 cells in the right flank. Three days following tumor cell implantation, palpable dark melanoma tumors appeared. $^{212}$Pb-DOTA-Re(Arg$^{11}$)CCMSH treatment was started on the 4th day after tumor cell inoculation. The effects of $^{212}$Pb-DOTA-Re(Arg$^{11}$)CCMSH treatment on tumor growth rate and mean body weight of the mice in the therapy study are shown in Fig. 2A and B, respectively. Single-dose administrations of 50, 100, and 200 μCi of $^{212}$Pb-DOTA-Re(Arg$^{11}$)CCMSH caused substantial tumor growth inhibition over the time period of the therapy study compared with the untreated tumor control group (Fig. 2A). Treated mice maintained healthy physical appearances (without diarrhea and scruff coat), normal activity levels (without anorexia and weight gain throughout the study period (Fig. 2B). Tumor response to $^{212}$Pb-DOTA-Re(Arg$^{11}$)CCMSH treatment was dose-dependent. Decreases in tumor growth rates were more pronounced for the 100 μCi treatment group compared with the 50 μCi treatment group. Two of 10 mice in the 100 μCi treatment group were tumor-free at the completion of the 120-day study. The treatment group receiving the 200 μCi of $^{212}$Pb-DOTA-Re(Arg$^{11}$)CCMSH exhibited the best overall tumor growth inhibition (Fig. 2A). Four of nine mice in the 200 μCi treated group survived the 120-day study tumor-free, whereas two of the nine mice displayed a partial tumor progression.

### Table 1. Pharmacokinetics of $^{212}$Pb-DOTA-Re(Arg$^{11}$)CCMSH in B16/F1 murine melanoma C57 mice

<table>
<thead>
<tr>
<th>Tissues</th>
<th>5 min</th>
<th>30 min</th>
<th>1 h</th>
<th>2 h</th>
<th>4 h</th>
<th>24 h</th>
<th>48 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor</td>
<td>13.49 ± 3.50</td>
<td>12.72 ± 1.96</td>
<td>13.33 ± 2.78</td>
<td>11.25 ± 1.52</td>
<td>12.84 ± 2.53</td>
<td>4.59 ± 1.45</td>
<td>3.02 ± 1.49</td>
</tr>
<tr>
<td>Brain</td>
<td>0.30 ± 0.05</td>
<td>0.12 ± 0.01</td>
<td>0.05 ± 0.02</td>
<td>0.09 ± 0.03</td>
<td>0.03 ± 0.05</td>
<td>0.02 ± 0.02</td>
<td>0.02 ± 0.02</td>
</tr>
<tr>
<td>Blood</td>
<td>8.20 ± 0.99</td>
<td>2.62 ± 0.72</td>
<td>0.74 ± 0.24</td>
<td>0.27 ± 0.08</td>
<td>0.08 ± 0.05</td>
<td>0.02 ± 0.03</td>
<td>0.02 ± 0.02</td>
</tr>
<tr>
<td>Heart</td>
<td>3.79 ± 0.57</td>
<td>1.41 ± 0.28</td>
<td>0.40 ± 0.15</td>
<td>0.33 ± 0.15</td>
<td>0.06 ± 0.04</td>
<td>0.03 ± 0.03</td>
<td>0.03 ± 0.03</td>
</tr>
<tr>
<td>Lung</td>
<td>8.15 ± 0.52</td>
<td>2.80 ± 0.84</td>
<td>1.11 ± 0.51</td>
<td>0.32 ± 0.04</td>
<td>0.40 ± 0.36</td>
<td>0.04 ± 0.03</td>
<td>0.07 ± 0.01</td>
</tr>
<tr>
<td>Liver</td>
<td>2.64 ± 0.55</td>
<td>1.16 ± 0.23</td>
<td>0.63 ± 0.14</td>
<td>0.42 ± 0.05</td>
<td>0.41 ± 0.13</td>
<td>0.19 ± 0.03</td>
<td>0.15 ± 0.01</td>
</tr>
<tr>
<td>Spleen</td>
<td>2.60 ± 0.29</td>
<td>1.54 ± 0.35</td>
<td>0.51 ± 0.10</td>
<td>0.42 ± 0.18</td>
<td>0.30 ± 0.22</td>
<td>0.09 ± 0.08</td>
<td>0.09 ± 0.05</td>
</tr>
<tr>
<td>Stomach</td>
<td>3.80 ± 0.44</td>
<td>1.23 ± 0.32</td>
<td>0.48 ± 0.13</td>
<td>0.20 ± 0.05</td>
<td>0.12 ± 0.06</td>
<td>0.14 ± 0.12</td>
<td>0.06 ± 0.03</td>
</tr>
<tr>
<td>Kidneys</td>
<td>32.91 ± 7.04</td>
<td>13.24 ± 2.81</td>
<td>9.52 ± 1.71</td>
<td>7.31 ± 1.26</td>
<td>4.56 ± 1.27</td>
<td>2.93 ± 0.53</td>
<td>1.98 ± 0.20</td>
</tr>
<tr>
<td>Muscle</td>
<td>1.91 ± 0.25</td>
<td>0.86 ± 0.28</td>
<td>0.39 ± 0.02</td>
<td>0.16 ± 0.04</td>
<td>0.05 ± 0.06</td>
<td>0.00 ± 0.00</td>
<td>0.02 ± 0.02</td>
</tr>
<tr>
<td>Pancreas</td>
<td>2.08 ± 0.11</td>
<td>0.85 ± 0.28</td>
<td>0.30 ± 0.09</td>
<td>0.18 ± 0.08</td>
<td>0.09 ± 0.04</td>
<td>0.07 ± 0.02</td>
<td>0.03 ± 0.01</td>
</tr>
<tr>
<td>Bone</td>
<td>4.61 ± 1.90</td>
<td>2.06 ± 0.76</td>
<td>0.94 ± 0.46</td>
<td>0.63 ± 0.24</td>
<td>0.36 ± 0.20</td>
<td>0.15 ± 0.07</td>
<td>0.32 ± 0.19</td>
</tr>
<tr>
<td>Skin</td>
<td>8.43 ± 1.05</td>
<td>5.33 ± 0.93</td>
<td>1.95 ± 0.82</td>
<td>0.42 ± 0.04</td>
<td>0.26 ± 0.13</td>
<td>0.06 ± 0.03</td>
<td>0.08 ± 0.03</td>
</tr>
<tr>
<td>Intestines</td>
<td>3.56 ± 0.12</td>
<td>1.44 ± 0.32</td>
<td>0.88 ± 0.43</td>
<td>0.60 ± 0.14</td>
<td>0.49 ± 0.04</td>
<td>0.14 ± 0.02</td>
<td>0.17 ± 0.15</td>
</tr>
<tr>
<td>Urine</td>
<td>54.98 ± 3.73</td>
<td>62.11 ± 4.35</td>
<td>80.14 ± 9.94</td>
<td>89.70 ± 2.15</td>
<td>94.50 ± 1.14</td>
<td>97.17 ± 0.74</td>
<td>97.08 ± 1.11</td>
</tr>
</tbody>
</table>

NOTE: The data are presented as the percentage of injected dose per gram or as a percentage of injected dose (mean ± SD, n = 4).
remission response at 35 and 55 days (Fig. 3). The mean survival time for the untreated tumor control group was 14.6 ± 4.4 days. The 50 μCi treated group exhibited a mean survival time of 22.0 ± 5.5 days, which was significantly longer than the untreated tumor control group (P = 0.004, Fig. 3). Mean survival times of tumor mice in the 100 and 200 μCi treatment groups that did not survive the entire study were significantly extended to 28.0 ± 8.8 (P = 0.002) and 49.8 ± 27.3 days (P = 0.02), respectively (Fig. 3). After completion of the therapy study, the kidneys and tumor implantation sites of the treated mice that survived the study were examined. Histopathologic examination showed evidence of moderate kidney toxicity, with a thinner than normal renal cortex and damage to glomeruli and tubular structures despite lack of observational effects during the study period (Fig. 4). No evidence of secondary tumor formation was found on necropsy. Biopsies of tumor implantation sites revealed the presence of macrophage infiltrates, but no melanoma cells. Staining for the melanoma antigen S100 was also negative, indicating that the mice that survived the study were tumor-free.

Discussion

In this study, the pharmacokinetics and therapeutic efficacy of 212Pb-labeled DOTA-Re(Arg11)CCMSH were determined in B16/F1 murine melanoma–bearing mice. The decay scheme of 212Pb includes 212Bi, which yields an α-particle, two β-particles, and several γ-emissions upon decay. α-Particle emitters are particularly attractive for targeted radiotherapy due to high linear energy transfer properties such as localized dense ionization, which results in irreparable DNA double-strand breaks and cytotoxicity that is independent of tissue oxygen content or dose rate (3). A major advantage of using 212Pb-DOTA-Re(Arg11)CCMSH is that 212Pb delivers >10 times the dose per unit of administered activity compared with either 212Bi or 211Bi alone (23). Peptide-targeted 212Pb, internalized and retained by tumor cells decays to the α-particle emitting 212Bi, localizing the highly toxic short-range α-radiation within the tumor. This strategy is referred to as an in vivo generator approach that produces a high dose delivery to the targeted melanoma tumor cells, whereas resulting in lower nonspecific irradiation to normal tissue. Moreover, the short half-life of 212Bi (t1/2 = 60.6 minutes) can be effectively extended by conjugating its longer-lived parent radionuclide of 212Pb (t1/2 = 10.6 hours) to DOTA-Re(Arg11)CCMSH. The relative long half-life of 212Pb provides sufficient time for 212Pb-DOTA-Re(Arg11)CCMSH dose preparation and administration with minimal loss of activity as well as minimizing losses during in vivo tumor targeting.

Biodistribution results in B16/F1 melanoma tumor–bearing mice showed that 212Pb-DOTA-Re(Arg11)CCMSH had high receptor-mediated tumor uptake and prolonged retention. Disappearance of activity from the normal organs and tissues was rapid. The majority of the administered activity was cleared through the kidneys, with ~90% of the injected dose being excreted in the urine by 2 hours postinjection. As seen with other radiolabeled peptides, disappearance of activity from the kidneys was slower than the other major organs. Coinjection of excess nonradioactive NDP-MSH peptide dramatically reduced tumor uptake but did not affect radioactivity in the kidneys, demonstrating that the kidney radioactivity is nonspecific and...
not mediated by MC1 receptor interaction. The infusion of lysine or arginine, prior to or with dose administration, has been successful in reducing nonspecific retention of activity in the kidneys (24). Coinjection of free lysine or arginine in this instance, however, did not affect kidney radioactivity levels of $^{212}$Pb-DOTA-Re(Arg$^{11}$)CCMSH. Changing lysine$^{11}$ to arginine in the CCMSH peptide significantly increased its tumor uptake and reduced its kidney retention (25). However, the amino acid substitution also eliminated additional suppression of nonspecific kidney uptake of radioactivity by lysine or arginine coinfusion. It is possible that radioactivity in the kidneys may be due to free $^{212}$Bi released during the decay of $^{212}$Pb or radioactive metabolites of $^{212}$Pb-DOTA-Re(Arg$^{11}$)CCMSH.

It was reported that approximately one-third of the $^{212}$Bi is lost from the DOTA chelator during the transition from $^{212}$Pb to $^{212}$Bi, due to transient oxidation states of the resulting bismuth that occur during the conversion process (26). Normal tissue toxicity of α-particle emitting radionuclides is a concern due to the high cytotoxicity of α-radiation. Dosimetry results showed that the tumor received the highest dose followed by the kidneys and blood. The toxicity of $^{212}$Bi circulating in blood is unlikely to be significant due to the short path length of α-radiation and short resonance time. However, radioactivity residing in the kidney may result in renal toxicity. Acute radiation nephritis was reported in Cynomolgus monkeys treated with escalating doses of an α-particle emitting $^{225}$Ac-HuM195 monoclonal antibody complex (27). Estimated kidney doses of 9 to 13 Gy to the renal cortex from $^{225}$Ac and its α-particle-emitting daughters, including $^{211}$Bi, resulted in tubular epithelial necrosis and marked regeneration. Accumulation of $^{213}$Bi or $^{212}$Bi in the kidneys was consistent with biodistribution studies of nonchelated or free radiobismuth (28).

Intravital chelation of $^{212}$Bi has been one method proposed to reduce radioactivity in the kidneys. DMPS intravital chelation of free $^{212}$Bi generated by circulating $^{212}$Pb radiolabeled antibodies was shown to reduce bismuth activity in the kidneys (29). The extended circulation time of the $^{212}$Pb radiolabeled antibodies would allow ample time for the generation of free $^{212}$Bi in vivo, allowing an intravital chelation strategy to be effective. However, the rapid pharmacokinetics of small radiolabeled peptides dramatically minimizes the amount of free $^{212}$Bi.

Fig. 3. Survival analysis of B16/F1 murine melanoma–bearing C57 mice. Kaplan-Meier survival curves for B16/F1 murine melanoma–bearing mice treated with 50 μCi (A), 100 μCi (B), and 200 μCi (C) of $^{212}$Pb-DOTA-Re(Arg$^{11}$)CCMSH or a saline placebo (D). Mean survival times were 14.6 ± 4.4 days for untreated tumor control group, 22.0 ± 5.5 days ($P = 0.004$) for 50 μCi treated group, 28.0 ± 8.8 days ($P = 0.002$) for 100 μCi treated group, and 49.8 ± 27.3 days ($P = 0.02$) for the 200 μCi treated group. Two out of 10 mice in the 100 μCi treated group and four out of nine mice in the 200 μCi treated group were free of tumor and survived the 120-day studies.

Fig. 4. Kidney histology sections from an untreated C57 mouse (A) and C57 mice treated with 100 μCi (B) and 200 μCi (C) of $^{212}$Pb-DOTA-Re(Arg$^{11}$)CCMSH 100 days post-dose administration. Bar, 0.5 mm.
introduction of negatively charged amino acids between DOTA and the recycelized peptide was shown to dramatically decrease nonspecific kidney retention of $^{177}$Lu-DOTA-Re(Arg$^{11}$)CCMSH (30). It is very likely that the renal uptake of $^{212}$Pb-DOTA-Re(Arg$^{11}$)CCMSH can be reduced by employing the same strategy in the future investigations. Furthermore, a multiple-dose radiotherapy regimen may result in a lower kidney dose, whereas maintaining high therapeutic efficacy (31–34).

The therapeutic efficacy of peptide-targeted α-therapy was greater than peptide-targeted $^{188}$Re β-emitter therapy in the B16/F1 solid tumor melanoma mouse model (31). Tumor-bearing mice treated with single 200 or 600 μCi doses of $^{188}$Re-(Arg$^{11}$)CCMSH complex exhibited no improvement in mean survival over the non–treatment control group. Only mice treated with 2 × 400 μCi doses of $^{188}$Re-(Arg$^{11}$)CCMSH displayed an improvement in mean survival. However, there were no complete remissions or durable cures. In the $^{212}$Pb-DOTA-Re(Arg$^{11}$)CCMSH therapy studies reported here, all treated groups of melanoma mice exhibited significant ($P < 0.05$, Fig. 3) improvement in mean survival over the non–treatment control group. Moreover, 20% and 45% of the mice receiving 100 or 200 μCi of $^{212}$Pb-DOTA-Re(Arg$^{11}$)CCMSH survived the study disease-free. The differences in therapeutic efficacies are likely due to the differences in radiation decay properties and tumor size. Rhenium-188 is a high-energy β-emitter, which may have resulted in much of its radiation being deposited outside the tumor volume. High-energy β-emitters, such as $^{188}$Re and $^{186}$Re, are probably better suited for large tumor volumes, as exemplified by targeted radiotherapy with radiolabeled octreotide analogues (35).

In this study, $^{212}$Pb-DOTA-Re(Arg$^{11}$)CCMSH treatment significantly decreased tumor growth rates and extended the mean survival time of melanoma-bearing mice in the B16/F1 murine melanoma model. Targeted radiotherapy with short path length high–linear energy transfer $^{212}$Bi α-particles focused energy deposition within tumor cells yielding dramatic improvements in melanoma therapeutic efficacies. $^{212}$Pb-DOTA-Re(Arg$^{11}$)CCMSH seems to be a very promising radio-pharmaceutical for targeted radionuclide therapy of melanoma.

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