DOE DE-SC0005738 Final Scientific Report

Grant Name: AlphaMed

Title: Targeted Therapy for Melanoma

Performance Period: 03/01/2011 – 02/28/2013

University of Missouri Subcontract

The research project entitled," Targeted Therapy for Melanoma," was focused on investigating the use of kidney protection measures to lower the non-specific kidney uptake of the radiolabeled Pb-DOTA-ReCCMSH peptide. Previous published work demonstrated that the kidney exhibited the highest non-target tissue uptake of the $^{212Pb/203}$Pb radiolabeled melanoma targeting peptide DOTA-ReCCMSH (1,2). The radiolabeled alpha-melanocyte stimulating hormone (α-MSH) peptide analog DOTA-Re(Arg^{11})CCMSH, which binds the melanocortin-1 receptor over-expressed on melanoma tumor cells, has shown promise as a PRRT agent in pre-clinical studies. High tumor uptake of ^{212}Pb labeled DOTA-Re(Arg^{11})CCMSH resulted in tumor reduction or eradication in melanoma therapy studies (2). Of particular note was the 20-50% cure rate observed when melanoma mice were treated with alpha particle emitter ^{212}Pb. However, as with most PRRT agents, high radiation doses to the kidneys where observed. To optimize tumor treatment efficacy and reduce nephrotoxicity, the tumor to kidney uptake ratio must be improved. Strategies to reduce kidney retention of the radiolabeled peptide, while not effecting tumor uptake and retention, can be broken into several categories including modification of the targeting peptide sequence and reducing proximal tubule reabsorption (3).

Specific Aims:

The Aims of the project were, 1) examine the affect of peptide modification on kidney uptake, 2 inhibition of proximal tubule uptake, and 3) radioprotectors. Uptake and kidney dose modeling were applied to the data to determine if the kidney uptake interventions were effective in reducing the overall residence time in the kidneys.

Aim 1: Peptide synthesis and sequence modification:

DOTA-ReCCMSH and analogs contain a C-terminal modified sequence (DOTA-Re-Gly-CCEH-dPhe-RWCRPV-nh-Pr) and a Gly-Lys protease sensitive site were synthesized using standard solid phase peptide synthesis. The crude peptides were purified by reverse phase HPLC and their identities were confirmed by LC-MS analyses.

Radiolabeled peptides were prepared using Pb-203 obtained from Lantheus Medical Imaging using published methods (1). The radiolabeled peptides were HPLC purified prior to use.

Normal CD-1 mice were injected with the radiolabeled peptide and sacrificed at 1 h, 4 h and 24 h post injection, dissected, and the major organs including the kidneys were weighed and counted for radioactivity. The data were reported as percent injected dose (% ID) or percent injected dose per gram (% ID/g).

C-terminal modification resulted in a dramatic increase in non-specific kidney retention. Kidney uptake was at 2 h, 4 h and 24 h post injection was 14.56±4.00% ID/g, 16.47±2.90% ID/g and 9.00±1.38% ID/g, respectively. This compared to the baseline non-specific kidney retention of 7.74±1.17% ID/g, 12.74±6.52% ID/g and 6.53±1.20% ID/g at the same time points. C-terminal modifications, which involve the addition of sequence was found to result in more kidney uptake.

Aim 2: Inhibition of proximal tubule reabsorption of radiolabeled peptide:

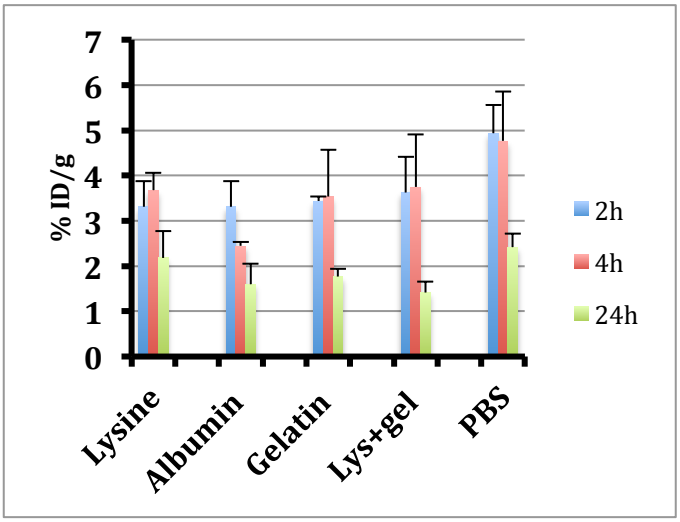

Figure 1. Kidney uptake of ^{203}Pb-DOTA-ReCCMSH in CD-1 normal mice (n=4) pre injected with lysine, albumin fragments, gelatin or Lys+gelatin at 1 h, 4 h, and 24 h post injection. Data are expressed in percent injected dose per gram of tissue.

Pre-injection of lysine (Lys) (4), albumin (5), gelatin (6) and a combination of Lys + gelatin (3,4,6) were examined for their abilities to reduce non-specific kidney uptake and retention of ^{203}Pb-DOTA-ReCCMSH. A PBS injection was also performed to simulate increased volume administration. Positively charged amino acids such al lysine and arginine have been shown to reduce non-specific radiolabeled peptide uptake (4). Recently, albumin (5) and succinylated gelatin (6) and were reported to decrease kidney uptake of radiolabeled peptides. Increased fluids iv were also examined to facilitate peptide clearance. Groups of 4 normal CD-1 mice were injected intravenously with lysine (400 mg/Kg), albumin fragments (250 mg/Kg) succinylated gelatin (80 mg/Kg), lysine + gelatin and PBS. The mice were sacrificed at 1 h, 4 h and 24 h post injection, dissected, and the major organs including the kidneys were weighed and counted for radioactivity. The data were reported as percent injected dose (% ID) or percent injected dose per gram (% ID/g). The data for the tubule reabsorption inhibitors are presented in Figure 1. All administered agents resulted in a reduction of kidney retention. Pre-administration of albumin fragments resulted in the best reduction of non-specific kidney uptake. Lysine, gelatin or a co-injection of lysine + gelatin resulted in similar reduction percentages. All of the kidney reduction interventions lowered non-specific kidney uptake and retention of ^{203}Pb-DOTA-ReCCMSH compared to direct administration of ^{203}Pb-DOTA-ReCCMSH (Fig 2). Albumin fragments had the greatest reduction in non-specific kidney uptake and retention integrated over the 24 h time period. Since albumin fragments are not homogenous there may be difficulty getting them approved. Since Lysine is already used in the clinic to reduce non-specific kidney uptake, and provides nearly identical protection, it seems reasonable to continue its use as apposed to having a new product approved. The use of succinylated gelatin or gelatin plus lysine did not provide as much protection as lysine or the albumin fragments. In summary, while albumin did reduce kidney retention slightly better than lysine it was not enough to warrant development of a new product. Positively charged amino acids like lysine are already used in the clinic. Succinylated gelatin was better than increased fluid load using PBS, but was not significantly better than lysine. The combination of Lys + gelatin did not show improvement.

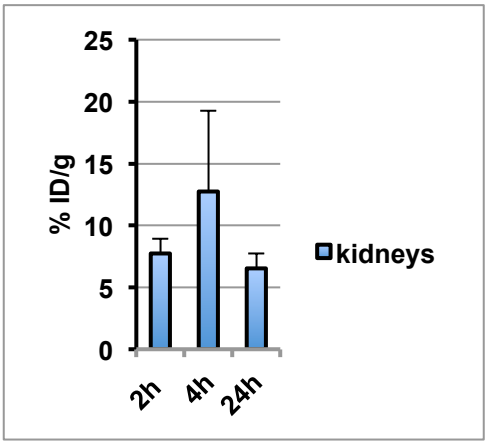

Figure 2. Kidney uptake of ^{203}Pb-DOTA-ReCCMSH in B16/F1 melanoma bearing C57 mice (n=4) at 1 h, 4 h, and 24 h post injection. Data are expressed in percent injected dose per gram of tissue.

Literature Cited:

1. Miao Y, et al. 203Pb-labeled alpha-melanocyte-stimulating hormone peptide as an imaging probe for melanoma detection. J Nucl Med. 2008 May;49(5):823-9.
2. Miao Y, et al. Melanoma therapy via peptide-targeted a-radiation. Clin Cancer Res. 2005;11:5616-21.
3. Vegt E, et al. Renal toxicity of radiolabeled peptides and antibody fragments: mechanisms, impact on radionuclide therapy, and strategies for prevention. J Nucl Med. 2010;51:1049-58.
4. Kobayashi, H, et al. l-Lysine Effectively Blocks Renal Uptake of 125I-or 99mTc-labeled Anti-tac Disulfide-stabilized Fv Fragment. Cancer Res. 1996;56:3788-95.
5. Vegt E, et al. Reducing renal uptake of radiolabeled peptides using albumin fragments. J Nucl Med. 2008;49:1506-11.
6. Melis, M., et al. Dose-response effect of Gelofusine on renal uptake and retention of radiolabelled octreotate in rats with CA20948 tumours. European Journal of Nuclear Medicine and Molecular Imaging. 2009;36:1968–1976.