^{111}In-Streptavidin-biotinylated α-melanocyte-stimulating hormone 2.0 bacteriophage

^{111}In-SA-α-MSH2.0 phage

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Chemical name:	^{111}In-Streptavidin-biotinylated α-melanocyte-stimulating hormone 2.0 bacteriophage	
Abbreviated name:	^{111}In-SA-α-MSH2.0 phage	
Synonym:	Pretargeted ^{111}In-MSH bacteriophage	
Agent Category:	Bacteriophage	
Target:	Melanocortin-1 (MC-1) receptor	
Target Category:	Receptor binding	
Method of detection:	Single-photon emission computed tomography (SPECT) or gamma planar imaging	
Source of signal/contrast:	^{111}In	
Activation:	No	
Studies:	• *In vitro* • Rodents	Click on protein, nucleotide (RefSeq), and gene for more information about the melanocortin-1 receptor.

Background

[PubMed]

^{111}In-Streptavidin-biotinylated α-melanocyte-stimulating hormone (MSH) 2.,0 bacteriophage (^{111}In-SA-α-MSH2.0 phage) is a two-step melanoma pretargeting and imaging system developed as a single-photon emission computed tomography (SPECT) imaging probe for primary and metastatic melanoma (1). This system uses a biotinylated

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α-MSH2.0 (bio-MSH2.0) targeting phage combined with *in vivo* labeling by ^{111}In-streptavidin (^{111}In-SA).

Malignant melanoma is the sixth most common cancer in the United States (2). Early diagnosis and prompt surgical removal comprise the best approach for treatment (3). The melanocortin (MC) system is the best characterized neuropeptide network of the skin, and it is involved in pigmentation regulation, cortisol production, and many other physiological processes (4). Most cutaneous cell types express MC receptors, proopiomelanocortin (POMC), and prohormone convertases, and they also release MCs. Five MC receptors (MC-1 to MC-5) have been cloned and characterized as receptors that belong to the G-protein–coupled receptor superfamily. MSHs (α-, β-, and γ-MSH) are derived from POMC by the proteolytic action of prohormone convertases. α-MSH (Ac-Ser^{1}-Tyr^{2}-Ser^{3}-Met^{4}-Glu^{5}-His^{6}-Phe^{7}-Arg^{8}-Trp^{9}-Gly^{10}-Lys^{11}-Pro^{12}-Val^{13}-NH_2), composed of 13 amino acids, is the most potent naturally occurring melanotropic peptide (5). The biological effects of α-MSH are mediated *via* MC receptors.

Although positron emission tomography imaging with [^{18}F]fluoro-2-deoxy-2-D-glucose ([^{18}F]FDG) is effective in the detection of melanoma, it is not melanoma-specific and some melanoma cells do not take up [^{18}F]FDG (6, 7). Radiolabeled α-MSH peptide analogs have been shown to specifically bind to MC-1 receptors that are overexpressed on human and mouse melanoma cells (6, 8-11). To improve the *in vivo* pharmacokinetics of these radiolabeled peptides, different α-MSH analogs have been designed and studied (12, 13). Bacteriophage display (phage display) is a combinatorial chemistry technique that uses a population of filamentous bacteriophage particles genetically modified to display a library of proteins and peptides on their surface (14, 15). This technique has been used for the discovery of new and unique molecular imaging peptides (16). These phage display–derived peptides can have binding properties different from chemically synthesized peptides because the conformational structures of phage peptides are affected by the microenvironment created by the surface of the phage particle (1). Instead of using the isolated peptides, Newton et al. (1) suggested that imaging tags can be directly added to phage particles without significant effects on their peptide binding properties. However, these very high molecular weight phage particles are generally cleared by the reticuloendothelial system *in vivo*. To circumvent this problem, Newton et al. (1) prepared an α-MSH2.0 peptide analog from the phage display library and proposed the use of a two-step biotin-SA pretargeting system to allow for clearance of unlabeled phage particles before injection of the ^{111}In imaging label.

Synthesis

[PubMed]

The α-MSH2.0 phage was generated by use of the fUSE5 vector, which contained phage with modified coat protein III and displayed up to 5 copies of a fused peptide on the tip of the phage particle (1). Affinity selection of phage constructs was performed through multiple rounds of washing and multiplication. Briefly, the fUSE5 vector was digested

with the *SFi*I restriction endonuclease. Equal molar amounts of sense and antisense phosphorylated DNA oligonucleotides were hybridized. A room-temperature DNA ligation reaction was performed for insertion of the hybridized DNA into the fUSE5 vector. *Escherichia coli* K91 Blue Kan cells were electroporated in the presence of the ligated vector. The proper DNA sequence was verified by DNA sequencing, and the amplified phage was precipitated. The phage particle concentrations in virions were determined spectrophotometrically.

Biotinylation of the phage particle was conducted by adding commercially available NHS-PEO_4-biotin to the phage suspension at a 1,000-fold molar excess relative to the phage particles (1). The mixture was rotated at room temperature for 2 h. The reaction was stopped with ethanolamine (pH 9.0) and was rotated at room temperature for 1 h. The free biotin was removed from bio-MSH2.0 by dialysis. ^{111}In-SA was prepared by first attaching diethylenetriaminepentaacetic acid (DTPA) to SA molecules. This was performed by mixing 2-(4-isothiocyanatobenzyl)-DTPA in carbonate buffer (pH 9.5) with SA suspension at a 50-fold molar excess of DTPA. The mixture was rotated overnight at 4°C. After removal of excess DTPA, DTPA-SA was radiolabeled with ^{111}In by incubation with ^{111}In chloride in 10 mM *N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid (HEPES, pH 7.0) at 37°C for 1.5 h. Free ^{111}In was removed by Zeba Desalt Spin Columns. The specific activity and radiochemical purity of ^{111}In-SA were not reported.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Newton et al. (1) conducted phage sequencing to confirm that the MSH2.0 phage clone contained a modified α-MSH sequence. MSH2.0 lacked 3 nonessential amino acids at the amino terminus of the α-MSH sequence and contained a linker peptide between it and the phage coat protein III (NH_2-AMEHFRWGRPVGSGSGSGSVWYAG-coat protein III). The phase clones were then probed with an anti–α-MSH antibody to determine whether the peptide sequences were accessible and reactive with the MC-1 receptor. The phage particle dot blot assay showed that the α-MSH sequence on the MSH2.0 phage clone was immunoreactive. A micropanning assay with B16/F1 mouse melanoma cells was performed to test the specific binding of MSH2.0 phage. The results indicated specific binding of MSH2.0 peptide to B16/F1 cells. There was no significant alteration of phage binding *in vitro* with the addition of PEO_4-biotin to the surface of the phage particle.

The DTPA-SA complex was prepared with >90% labeling efficiency (1). An *in vitro* stability test showed that ^{111}In-SA retained 100% labeling activity in phosphate-buffered saline and HEPES for at least 24 h at 25°C and 37°C. ^{111}In-SA retained ~80% activity when incubated in mouse serum for 24 h.

Animal Studies

Rodents

[PubMed]

Newton et al. (1) conducted biodistribution studies of the pretargeted bio-MSH2.0 followed by ^{111}In-SA in mice bearing B16/F1 mouse melanoma (0.05–0.1 g s.c. tumors). Each mouse received an i.v. injection of 5×10^{12} virions of bio-MSH2.0. After 4 h, an i.v. injection of 1.85 MBq (0.05 mCi) ^{111}In-SA was administered. ^{111}In-SA radioactivity was primarily cleared by the urinary and hepatobiliary systems. The initial tumor radioactivity of ~2.5% injected dose per g (% ID/g; $n = 3$) appeared to be attributable to the tumor blood volume. After 24 h, there was actual radioactivity retention of 1.0 ± 0.1% ID/g and the tumor/muscle and tumor/blood ratios were 17.5 ± 3.7 and 2.0 ± 0.2, respectively. In comparison, the tumor radioactivity of ^{111}In-SA alone without bio-MSH2.0 was 0.4 ± 0.1% ID/g at 24 h. The biotinylated nonspecific wild-type phage tumor radioactivity level was 1.8-fold less than that of bio-MSH2.0 at 24 h. This suggested that the *in vivo* targeting of ^{111}In-SA-α-MSH2.0 phage to the tumor was mediated by α-MSH peptide. Coinjection of bio-MSH2.0 with 100 μg of [NIe^4,D-Phe^7]α-MSH (NDP), a potent protease-resistant peptide analog of α-MSH, decreased the tumor radioactivity retention of bio-MSH2.0 by 2.4 ± 0.4-fold. The authors suggested that this demonstrated the specificity of binding for the bio-MSH2.0 phage *in vivo*.

SPECT imaging of ^{111}In-SA–α-MSH2.0 phage was performed in mice bearing B16/F1 mouse melanoma (1). Each mouse received 5×10^{12} virions of bio-MSH2.0 and then 7.4 MBq (0.2 mCi) of ^{111}In-SA. The study clearly demonstrated the accumulation of ^{111}In-SA radioactivity within the tumor. The mice were euthanized 4 h after injection. The tumor radioactivity level at 4 h was 5.2 ± 0.8% ID/g, which was higher than the biodistribution study value of 0.9 ± 0.3% ID/g. The authors suggested that the higher value in the imaging study was attributable to the higher dose of ^{111}In-SA administered in the study.

Other Non-Primate Mammals

[PubMed]

No publication is currently available.

Non-Human Primates

[PubMed]

No publication is currently available.

Human Studies

[PubMed]

No publication is currently available.

NIH Support

NIH P50 CA103130-01.

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