Poly(ethylene glycol)-coated gold nanocages bioconjugated with [Nle^4,D-Phe^7]-α-melanotropin-stimulating hormone

[Nle^4,D-Phe^7]-α-MSH-PEG-AuNCs

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Chemical name:	Poly(ethylene glycol)-coated gold nanocages bioconjugated with [Nle^4,D-Phe^7]-α-melanotropin-stimulating hormone	
Abbreviated name:	[Nle^4,D-Phe^7]-α-MSH-PEG-AuNCs	
Synonym:		
Agent category:	Peptide	
Target:	Melanocortin-1 receptor, MC1R	
Target category:	Receptor	
Method of detection:	Ultrasound, photoacoustic tomography (PAT) imaging	
Source of signal:	Gold (Au)	
Activation:	No	
Studies:	• *In vitro* • Rodents	Click on protein, nucleotide (RefSeq), and gene for more information about the melanocortin-1 receptor.

Background

[PubMed]

Optical fluorescence imaging is increasingly used to monitor biological functions of specific targets in small animals (1-3). However, the intrinsic fluorescence of biomolecules

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poses a problem when fluorophores that absorb visible light (350–700 nm) are used. Near-infrared (NIR) fluorescence (700–1,000 nm) detection avoids the background fluorescence interference of natural biomolecules, providing a high contrast between target and background tissues. NIR fluorophores have a wider dynamic range and minimal background as a result of reduced scattering compared with visible fluorescence detection. They also have high sensitivity, resulting from low infrared background, and high extinction coefficients, which provide high quantum yields. The NIR region is also compatible with solid-state optical components, such as diode lasers and silicon detectors. NIR fluorescence imaging is becoming a noninvasive alternative to radionuclide imaging in small animals (4, 5).

Photoacoustic imaging (PAI) is an emerging hybrid biomedical imaging modality based on the photoacoustic effect. In PAI, non-ionizing optical pulses are delivered into biological tissues. Some of the delivered energy is absorbed and converted into heat, leading to transient thermoelastic expansion and thus ultrasonic emission. The generated ultrasonic waves are then detected by ultrasonic transducers to form images. It is known that optical absorption is closely associated with physiological properties, such as hemoglobin concentration and oxygen saturation. As a result, the magnitude of the ultrasonic emission (i.e., photoacoustic signal), which is proportional to the local energy deposition, reveals physiologically specific optical absorption contrast and tissue structures. However, exogenous NIR contrast agents are necessary to overcome the intrinsic low tissue- and hemoglobin- absorption and scattering of tissue. On the other hand, these small molecules exhibit fast clearance, small optical absorption cross section, and non-targeted specificity. Therefore, there is a need for contrast agents with long blood circulation and targeted specificity.

Gold (Au) nanoparticles have been studied as molecular imaging agents because of their bright NIR fluorescence emission of 700–900 nm and low toxicity (6, 7). They can be tuned to emit in a range of wavelengths by changing their sizes, shapes, and composition, thus providing broad excitation profiles and high absorption coefficients. They can be coated and capped with hydrophilic materials for additional conjugation with biomolecules, such as peptides, antibodies, nucleic acids, and small organic compounds for *in vitro* and *in vivo* studies. Au nanoparticles have been approved by the United States Food and Drug Administration for the treatment of patients with rheumatoid arthritis. Au nanoparticles have been studied as contrast agents in X-ray/computed tomography, NIR optical coherence tomography, PAI, and photoacoustic tomography (PAT) (8). NIR Au nanocages (AuNCs) are biocompatible, have low toxicity, and are tunable to strong NIR absorption (9). They have an outer edge of ~50 nm and an inner edge of ~42 nm, with a wall thickness of ~4 nm. Yang et al. (10) have performed PAT of the cerebral cortex of rats with poly(ethylene glycol)-coated AuNCs (PEG-AuNCs) as an optical contrast agent. The investigators observed an enhanced optical contrast in the vasculature in the cerebral cortex. Song et al. (11) demonstrated the use of Au nanocages as a PAI probe for detection of sentinel lymph nodes in rats.

Malignant melanoma is the deadliest form of skin cancer (12). Early and accurate diagnosis is necessary for surgery and successful treatment (13). The melanocortin (MC) system is a neuropeptide network of the skin, and it is involved in pigmentation regulation, cortisol production, and many other physiological processes (14). Most cutaneous cell types express MC receptors, proopiomelanocortin (POMC), and prohormone convertases, and they also release MCs. However, these receptors have been found to be overexpressed in melanoma cells. There are five MC receptors (MC1R to MC5R), which belong to the G-protein–coupled receptor superfamily. Melanotropin-stimulating hormones (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), produced by the brain and pituitary gland, is a tridecapeptide (13 amino acids) and is the most potent melanotropic peptide (15) in the regulation of skin pigmentation *via* MC1R. Radiolabeled α-MSH peptide analogs have been shown to specifically bind to MC1R, which is overexpressed on human and mouse melanoma cells (16-20). Kim et al. (21) have evaluated [Nle^4,D-Phe^7]-α-MSH-PEG-AuNCs for *in vivo* PAT imaging of melanomas in mice.

Related Resource Links:

- Chapters in MICAD (MC1R, Gold imaging)
- Gene information in NCBI (MC1R)
- Articles in OMIM (MC1R)
- Clinical trials (MC1R, Gold nanoparticles)
- Drug information in FDA (Gold nanoparticles)

Synthesis

[PubMed]

Kim et al. (21) reported the synthesis of PEG-AuNCs by incubation of $HAuCl_4$ (0.5 mM) and Ag nanocubes containing poly(vinyl pyrrolidone) (PVP, 0.1 mg/ml) with stirring for 10 min at 100°C. Upon cooling to room temperature, excess NaCl was added to remove Ag as AgCl. The supernatant was discarded and the AuNCs were resuspended in water. The AuNC alloy was composed of 70% Au and 30% Ag. AuNCs were washed six times to remove PVP and NaCl. AuNC surfaces were functionalized with PEG by adding 1 mL of a 1-mM succinimidyl propionyl PEG-SH (5 kDa) to AuNCs. The suspension was incubated for 8 h at room temperature. Residual succinimidyl propionyl PEG-SH was removed by centrifugation. [Nle^4,D-Phe^7]-α-MSH was then incubated with a suspension of the AuNCs for 12 h at 7°C. [Nle^4,D-Phe^7]-α-MSH-PEG-AuNCs were isolated with centrifugation. AuNCs have an outer edge of ~46 nm and a wall thickness of ~7 nm as estimated with transmission electron microscopy. The number of [Nle^4,D-Phe^7]-α-MSH moieties per AuNC was not reported. [Nle^4,D-Phe^7]-α-MSH-PEG-AuNCs and PEG-AuNcs had maximum optical absorption at ~800 nm with a detection limit of 4.5 pM (Au) with PAI.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

B16-F0 mouse melanoma cells accumulated 123 ± 11 and 633 ± 121 [Nle^4,D-Phe^7]-α-MSH-PEG-AuNCs/cell (measured with inductively coupled plasma mass spectrometry (ICP-MS)) after 6 h and 24 h of incubation with 0.01 nM AuNCs (21), respectively. On the other hand, B16-F0 mouse melanoma cells accumulated 35 ± 30 and 182 ± 10 PEG-AuNCs/cell after 6 h and 24 h of incubation, respectively.

Animal Studies

Rodents

[PubMed]

Kim et al. (21) performed noninvasive PAT of mice (n = 4) bearing B16-F0 melanoma after intravenous injection of a single dose (1 nmol) of [Nle^4,D-Phe^7]-α-MSH-PEG-AuNCs or PEG-AuNcs. PAT scanning began immediately after injection and continued for ~6 h with the laser wavelength at 778 nm and ultrasound frequency of 10 MHz. PAT images revealed a greater optical contrast in the tumor after [Nle^4,D-Phe^7]-α-MSH-PEG-AuNCs injection compared with before injection. There was a gradual enhancement of the PA signal in the tumor with a maximum value of 38 ± 6% at 6 h after injection. In mice injected with PEG-AuNCs, there was a maximum enhancement of 13 ± 2% at 6 h after injection. Therefore, tumor accumulation of [Nle^4,D-Phe^7]-α-MSH-PEG-AuNCs was significantly higher than that of PEG-AuNCs (P = 0.007). *Ex vivo* ICP-MS analysis showed that the average number of [Nle^4,D-Phe^7]-α-MSH-PEG-AuNCs per tumor mass was 3.6 ± 1.0 × 10^8 AuNCs/g, whereas with PEG-AuNCs there were only 1.0 ± 1.0 × 10^8 AuNCs/g (P = 0.02). No blocking experiment was performed.

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.

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