HiLyte Fluor 647-hyaluronic acid-gold nanoparticles

HHAuNPs

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Chemical name:	HiLyte Fluor 647-hyaluronic acid-gold nanoparticles	
Abbreviated name:	HHAuNPs	
Synonym:		
Agent category:	Glycosaminoglycan	
Target:	Hyaluronidase (HAdase)	
Target category:	Enzyme	
Method of detection:	Optical, near-infrared (NIR) imaging	
Source of signal:	HiLyte Fluor 647	
Activation:	Yes	
Studies:	• *In vitro* • Rodents	No structure is currently available in PubChem.

Background

[PubMed]

Optical fluorescence imaging is increasingly used to visualize biological functions of specific targets in small animals (1, 2). However, the intrinsic fluorescence of biomolecules poses a problem when fluorophores that absorb visible light (350–700 nm) are used. Near-infrared (NIR) fluorescence (NIRF; 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

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compatible with solid-state optical components, such as diode lasers and silicon detectors. NIRF imaging is becoming a non-invasive alternative to radionuclide imaging in small animals.

Extracellular matrix (ECM) consists of a complex network of fibronectins, collagens, chondroitins, laminins, glycoproteins, hyaluronate, heparin sulfate, tenascins, and proteoglycans that surround connective tissue cells, and they are mainly secreted by fibroblasts, chondroblasts, and osteoblasts (3). ECM molecules are considered essential regulators of cell migration, differentiation, and tissue integrity and remodeling. These molecules play a role in inflammation and atherogenesis, but they also participate in the process of invasion and metastasis of malignant cells in the host tissue (4). Invasive tumor cells adhere to the ECM, which provides a matrix environment for permeation of tumor cells through the basal lamina and underlying interstitial stroma of the connective tissue.

Gold nanoparticles (AuNPs) have been used as X-ray and optical contrast agents in small animals with little toxicity (5). By combining AuNPs and fluorophore, the versatility of Au-fluorophore interactions allows detection of the presence of the AuNPs with fluorescence spectroscopy using NP surface energy transfer (NSET) (6, 7). NSET provides low noise/signal ratios and covers a distance of ~20 nm for metal–fluorophore interactions. Deep tissue imaging can be obtained with the use of NIR dyes. Hyaluronic acid (HA, also known as hyaluronan or hyaluronate) is a high molecular weight linear glycosaminoglycan widely distributed throughout connective, epithelial, and neural tissues and is a hyaluronidase (HAdase) substrate (8-10). The receptor for HA is CD44. HA provides a target for CD44-mediated adhesion of normal and cancer cells (11, 12). Elevated extracellular levels of HA and its partially catabolized oligomers are found in certain malignancies and inflammation (13, 14). Lee et al. (15) conjugated HA with NIR dye HiLyte Fluor 647 to AuNPs (HHAuNPs) for noninvasive detection of reactive oxygen species (ROS) and HAdase activity.

Synthesis

[PubMed]

Lee et al. (15) produced AuNPs (16 nm) using methods previously reported. Oligo-HA (3–8 kDa) and cystamine hydrochloride were dissolved in borate buffer and incubated with $NaBH_3CN$ for 5 d at 40°C. The mixture was incubated with dithiothreitol for 12 h to generate free thio groups (87%). End thiol modification of oligo-HA was activated with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide and reacted with HiLyte Fluor 647 amine to form HiLyte Fluor 647-oligo-HA (HH), which was conjugated to AuNPs to form HHAuNPs. There were 4.5 dye molecules per oligo-HA molecule and 30.9 molecules of oligo-HA per AuNP. The optical properties of HHAuNPs were stable in buffers with pH values 2–12 and serum (Ex/Em, 650/675 nm). The quenching efficiency was estimated to be >96%, mainly caused by the NSET between HH molecules and AuNPs.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Lee et al. (15) showed that HHAuNPs were activated by ROS (0.1 mM) such as superoxide and hydroxyl radical, as well as HAdase (200 U/ml), with >100-fold increases in NIRF signal after 3 h of incubation. ROS and HAdase caused degradation of the immobilized oligo-HA and thus released the quenching between HA molecules and AuNPS. Lipopolysaccharide and phorbol myristate acetate stimulate THP-1 cells to produce ROS, resulting in 1- to 3-fold increases in fluorescence signals. Cancer cell lines (HTC-116 and OVCAR-3) generated 4- to 6-fold increased fluorescence signals compared with a non-cancer cell line (NIH-3T3). This increase may be the result of cellular secretion of HAdase into the culture medium and the presence of HA receptor (CD44) on the cancer cells.

Animal Studies

Rodents

[PubMed]

Lee et al. (15) performed whole-body fluorescence imaging after HHAuNPs (0.5 pmol) were intra-articularly injected into the left forelimbs of both healthy and arthritic mice (n = 4). The NIRF signal was negligible in both healthy and arthritic joints at 30 min after the injection. However, a clearly detectable NIRF signal was observed only in arthritic joints at 2 h after injection. This NIRF signal peaked at 3 h and started to decrease over time. The level of NIRF in the arthritic joint was enhanced ~3.6-fold at 3 h as compared with levels at 30 min after injection. In contrast, no detectable NIRF changes were observed in healthy joints for 12 h. Intravenous injection of HHAuNPs (10 pmol) also showed a 5-fold enhancement in the inflamed regions of the arthritic joints at 6 h after injection. NIRF imaging in mice bearing subcutaneous human ovarian carcinoma (OVCAR-3) tumors detected a NIRF signal near the tumor sites at 6 h after intravenous injection. NIRF signal intensity continued to increase, with a 7.7-fold enhancement near the tumor sites and a 4.8-fold enhancement in the core of tumor at 24 h. *Ex vivo* examination of Au content and NIRF intensity in the tumor and in five major organs (the liver, spleen, lung, heart, and kidney) revealed that AuNPs and HHAuNPs exhibited a similar distribution pattern except in the tumor, where AuNPs and HHAuNPs resulted in Au levels of 10 and 80 ppm/mg, respectively; the liver had the highest Au content (>120 ppm/mg). On the other hand, the tumor produced the highest NIRF signal, which was 5-fold greater than the liver, which exhibited the second highest NIRF signal. This increase in NIRF signal may be the result of secretion of HAdase into the tumor microenvironment and the presence of the HA receptor (CD44) on the cancer cells. No blocking experiments were 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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