Cy5.5-Conjugated glycol chitosan-5β-cholanic acid nanoparticles

Cy5.5-CNPs

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Chemical name:	Cy5.5-Conjugated glycol chitosan-5β-cholanic nanoparticles	
Abbreviated name:	Cy5.5-CNPs	
Synonym:		
Agent Category:	Nanoparticles	
Target:	Non-targeted	
Target Category:	Non-targeted	
Method of detection:	Optical imaging (near-infrared fluorescence (NIRF) imaging)	
Source of signal / contrast:	Cy5.5	
Activation:	No	
Studies:	• *In vitro* • Rodents	Structure not available in PubChem.

Background

[PubMed]

Researchers have developed and are in the process of evaluating a variety of nanoparticles (NPs) that can be structurally customized to deliver imaging agents or antineoplastic drugs for the detection and/or treatment of various cancers (1). The main advantage of using NPs to deliver an imaging probe or a therapeutic drug is that, compared to conventional delivery options, NPs can transport larger amounts of a payload, within the probe or on its surface, because they have a high surface area to volume ratio (1, 2). Although the size of the NPs determines its circulation time and the route of excretion

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from the body (NPs <10 nm in size are removed through the kidneys and those that are 10–200 nm are taken out of circulation by the reticuloendothelial system), the chemical composition of an NP affects its functions *in vivo*; for more information on NPs, see Jokerst and Gambhir (3) and Fox et al. (4). Small sized NPs that are cleared from blood circulation may pass through gaps of 2–6 nm that are present in the vascular endothelium and accumulate to some extent in healthy tissues, but they are eventually removed by the lymphatic system and returned into blood circulation. Compared to normal tissues, solid tumors show a much higher uptake and prolonged retention of the NPs (known as the enhanced permeation and retention effect (EPR); for details, see Reddy (5)) because the vasculature in these lesions is leaky due to the presence of large pores (up to 1 μm) (4). In addition, reduced blood flow through the neoplastic lesion and an impaired lymphatic system leads to very little removal of NPs accumulated in the tumor (4). However, *in vivo* studies have shown that some types of NPs may not have optimal biodistribution in the body and tend to accumulate in tumors because of their surface chemistry, charge, size, etc.

NPs made of glycol chitosan-5β-cholanic acid, a water-soluble, biocompatible, biodegradable, and self-aggregating carbohydrate polymer (CNPs), have been shown in different models to have good potential to target therapeutic drugs to tumors (6). Factors considered important for the use of NPs to carry and deliver imaging agents or drugs to tumors are stability (for extended circulation), deformability (to avoid unwanted penetration and accumulation in angiogenic blood vessels), and the ability to be taken up rapidly by tumor cells (6). For the CNPs, these parameters have been studied primarily *in vitro*, and no studies have been performed to investigate these characteristic of the particles under *in vivo* conditions (6). The CNPs were labeled with Cy5.5, a near-infrared (NIRF) dye, and the labeled NPs (Cy5.5-CNPs) were investigated for their stability, deformability, and rapid uptake in tumor cells and in mice bearing neoplastic lesions on the flank (established with human squamous cell carcinoma SCC7 cells), brain (established with glioblastoma U87 cells), liver (established with murine colon cancer CT26 cells), and metastatic tumors (established with RFP-B16F10 cells; mouse melanoma cells transfected with the red fluorescent protein (RFP) gene) (6).

Related Resource Links

Nanoparticle-related chapters in MICAD

Chitosan-related chapters in MICAD

Fluorescent dye–related chapters in MICAD

Optical imaging–related chapters in MICAD

Synthesis

[PubMed]

The synthesis of glycol chitosan-5β-cholanic acid and its labeling with Cy5.5 have been described by Na et al. (6). Each molecule of glycol chitosan was reported to have 150 ± 4.5 molecules of 5β-cholanic acid as determined with a colloidal titration method. Measurement of the extinction coefficient of Cy5.5 in the final preparation of Cy5.5-CNPs showed that there were 4.8 ± 0.7 molecules of Cy5.5 attached to each molecule of glycol chitosan-5β-cholanic acid. Dynamic light scattering analysis and transmission electron microscopy of CNPs dissolved in distilled water or in phosphate-buffered saline (pH 7.4) showed that they self-assembled into spherical NPs with an average diameter of 264 ± 30 nm (6).

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

It has been shown that, compared with 90% of the Cy5.5-conjugated CNPs that could pass through a 0.2-μm filter membrane, most of the smaller polystyrene NPs (PSs; diameter = 210 ± 10 nm) could not pass through the membrane (6). This indicated that the Cy5.5-NPs were partially deformable compared with the PSs. This phenomenon could be repeated even after the Cy5.5-NPs were incubated in a 0.15-μM solution of human serum albumin (HSA) for 1 day at 37°C. This indicated the Cy5.5-NPs were stable in HSA for at least 24 h at physiological temperature.

The uptake of PSs and Cy5.5-NPs by HUVEC cells (a human umbilical vein endothelial cell line), RAW264.7 cells (a murine macrophage cell line), and SCC7 cells, respectively, was compared by Na et al. (6). Fluorescence microscopy showed that all cell lines took up the PSs, and maximum accumulation of these particles was observed in the RAW264.7 cells. The higher uptake of the labeled NPs by the RAW264.7 cells was attributed to the phagocytic nature of these cells. A low uptake of Cy5.5-NPs was observed in the HUVEC and RAW264.7 cells, but the NPs showed a rapid and high accumulation in the cytosol of the SCC7 tumor cells (within 30 min after exposure), indicating that the NIRF dye conjugated particles probably have an ability to specifically target tumor cells.

Animal Studies

Rodents

[PubMed]

To investigate the *in vivo* uptake of NPs by neoplastic tumor tissues, anesthetized athymic nude mice (n = 5 animals/group) bearing SCC7 cell lesions on the flank were injected with a solution of either Cy5.5-NPs (5 mg/kg body weight) or PSs (5 mg/kg body weight) through the tail vein (6). Subsequently, whole-body NIRF imaging was performed on the animals as described by Na et al. (6). At 1 h postinjection (p.i.), a strong fluorescence was observed throughout the body of the mice injected with the Cy5.5-NPs. By 1 day p.i., the tumors on the flank of these animals were clearly distinguishable from the surrounding normal tissue, indicating that the Cy5.5-NPs had the ability to specifically target the

tumors in the rodents. A histological examination of the tumors and the normal surrounding tissues of the animals showed that the NPs were present only in the tumor sections. This observation confirmed the results obtained from the NIRF images described above. In addition, the photon count per gram of *ex vivo* tumor tissue was 35-fold higher than that of other organs, including the liver and the spleen. By comparison, the PSs accumulated primarily in the liver of the mice and had a very low NIRF signal in the tumor. The Cy5.5-Nps, compared with the PSs, were reported to have a much longer blood circulation time (6).

Real-time intravascular dynamic NIRF imaging of mice injected with the Cy5.5-NPs or the PSs (n = 5 experiments) showed that the two particle types behaved differently in the vascular tissues of the animals (6). The dye-conjugated NPs were observed to move rapidly through the vasculature, and by 5 min p.i. these particles were located primarily in the tumor blood vessels. Over time, the Cy5.5-NPs showed a high rate of diffusion and accumulation into the deeper parts of the lesion. This behavior was not apparent in the normal blood vessels (e.g., in the colon tissue) and showed that rapid permeation of the Cy5.5-NPs at the angiogenic sites such as those in a tumor was a characteristic feature of the labeled NPs. By comparison, most of the PSs were cleared from circulation and accumulated in the liver of the rodents.

The brain tumor targeting specificity of Cy5.5-NPs was evaluated in anesthetized mice (n = 5 animals) bearing U87 glioblastoma cell tumors in the organ and on the dorsal flank as described by Na et al. (6). Control animals were injected with saline in the brain and had no tumors on the flank. The rodents were injected with Cy5.5-NPs as before, and whole-body NIRF images were acquired at 1, 14, and 21 days p.i. Although the tumors on the flank were clearly visible at 1 day p.i., the brain tumor could not be distinguished from the surrounding tissue at this time point. At 14 days p.i., a clear NIRF signal was obtained from both the tumors in the test mice, and the intensity of the signal was reported to peak in both the tumors by 21 days p.i. No NIRF signal was visible in the control mice. This demonstrated that growth of the tumor resulted in partial breakdown of the blood–brain barrier, which allowed vasculature-specific penetration of the NPs into the brain lesions. These observations were confirmed with *ex vivo* NIRF imaging of the tumors obtained from the brain and the flank of the mice.

In a liver tumor model (n = 5 mice bearing CT26 cell tumors in the right lobe of the organ), the investigators showed that the Cy5.5-NPs accumulated specifically in the tumors and that the NIRF signal from the lesions increased from 1 h to 24 h p.i (6). *Ex vivo* NIRF imaging and histological examination of the liver showed that the fluorescence signal was intense only on the right side of the organ and not in the whole organ. This indicated that the labeled NPs could avoid the reticuloendothelial system and accumulated primarily in the tumor cells of the liver.

To distinguish metastatic tumor cells from normal cells, anesthetized nude mice (n = 5 animals) bearing RFP-B16F10 cell tumors were injected with the fluorescent CNPs as before (6). Whole-body NIRF imaging of the animals showed that the fluorescence signal

was generated only from the lung region of the mice. Analysis of the fluorescence signal with z-stack imaging showed that the labeled NPs were localized and spread in the whole organ. *Ex vivo* analysis of the lungs showed there was a high expression of the RFP in the organ, and NIRF imaging showed accumulation of the Cy5.5-NPs in the lungs, thus confirming the presence of metastatic cancer in the tissue.

From these studies, the investigators concluded that Cy5.5-CNPs have a high stability and deformability and could be taken up rapidly by tumor cells in rodents (6).

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.

Supplemental Information

[Disclaimers]

No information is currently available.

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