Pegylated bis(4-(*N*-(2-naphthyl)phenylamino)phenyl)-fumaronitrile (NPAPF) nanoparticles

NPAPF-PEG NPs

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Chemical name:	Pegylated bis(4-(*N*-(2-naphthyl)phenylamino)phenyl)-fumaronitrile (NPAPF) nanoparticles	
Abbreviated name:	NPAPF-PEG NPs	
Synonym:		
Agent Category:	Nanoparticles	
Target:	Non-targeted	
Target Category:	Non-targeted	
Method of detection:	Optical imaging; near-infrared fluorescence imaging	
Source of signal / contrast:	bis(4-(*N*-(2-naphthyl)phenylamino)phenyl)-fumaronitrile (NPAPF)	
Activation:	No	
Studies:	• *In vitro* • Rodents	Structure not available in PubChem.

Background

[PubMed]

Free organic near-infrared fluorescent (NIRF) dyes such as indocyanine green are usually used as nontargeted molecular imaging probes (e.g., for lymph node mapping or angiography). However, these compounds are highly hydrophobic, have low stability under *in vivo* conditions, and are not considered suitable for the detection of cancerous

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tumors due to their low quantum yields (QY) after excitation (1). To circumvent these problems, the organic NIRF dyes are often covalently linked to or enclosed in nanoparticles (NPs; nanocarriers or nanoprobes) for delivery into the circulatory system, but the fluorescence signals generated from these preparations are quenched even when high concentrations of the dyes are enclosed in the nanocarriers because these the chemicals tend to aggregate (2). Therefore, the amount of NIRF dye that can be loaded into NPs is rather limited. An ideal NIRF dye should have a characteristic high brightness, show little or no aggregation at high concentrations, and have a high QY, and the signal generated from the compound should be easily detected even when it is encapsulated in the NPs (2). Conversely, some NIRF dyes exhibit very low or no fluorescence while in solution, but on aggregation emit a strong fluorescence signal (the phenomenon is called "aggregation-induced emission" (AIE) and has been explained in detail by Hong et al. (3)). Although AIE dyes have been shown to be suitable for the detection of proteins, DNA, etc., or for the staining of living cells, they have not been evaluated for the *in vivo* imaging of organs or tissues in animals or humans (3).

NPs may be made of either inorganic materials (e.g., quantum dots; silica or gold or iron oxide) or organic materials (e.g., polymer core NPs, polymer or lipid micelles, liposomes, etc.) as described in detail by Merian et al. (4). Organic NPs have often been loaded with NIFR dyes, conjugated with a targeting molecule (such as an antibody, receptor ligand, or a molecule that has an affinity for a specific component, usually on the cell membrane), and used to detect or monitor the treatment of neoplastic tumors, or to noninvasively visualize one or more organs or areas of interest in animals or humans (4). On the basis of the above information, Yang et al. used bis(4-(*N*-(2-naphthyl)phenylamino)phenyl)-fumaronitrile (NPAPF), a NIRF dye with AIE properties, to prepare NPs (NPAPF NPs), and then the investigators functionalized the nanoprobes with polyethylene glycol (NPAPF-PEG NPs) (2). To investigate the *in vitro* targeting properties of the NPs, they were conjugated to folic acid (NPAPF-PEG-FA NPs), and the targeted nanoprobes were used to visualize human epithelial cells (KB cells) under fluorescence microscopy. The biodistribution and imaging characteristics of the NPAPF-PEG NPs were studied in mice bearing human lung carcinoma cell line tumors (A549 cells).

Related Resource Links

Related chapters in MICAD

Human folate receptor protein and nucleotide sequences

Folate receptor–related clinical trials

Folate receptors 1, 2 and 3 in Online Mendelian Inheritance in Man database (OMIM)

Human folate receptor 2 in Gene database

Synthesis

[PubMed]

The synthesis and pegylation of NPAPF NPs (to obtain the NPAPF-PEG NPs) have been described by Yang et al. (2). Conjugation of the NPs with FA is detailed elsewhere (2). The number of PEG or FA molecules attached to each NPAPF NP or NPAPF-PEG NP, respectively, was not reported. The NPAPF NPs were reported to have a nearly spherical shape, with a size distribution range of 60–100 nm as determined with scanning electron microscopy (2). The emission peak of the NPs was at a wavelength of 650 nm, and the QY of the nanoprobes in water was determined to be up to 14.9%.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

The effects of phosphate-buffered saline (PBS; pH not mentioned), serum (source not reported), and pH ranging from 4 to 10 on the NPAPF NPs and NPAPF-PEG NPs were investigated by Yang et al. (2). The PBS, serum, or solutions with the different pH values did not affect the size of the NPAPF-PEG NPs for up to at least 48 h. However, during the same period, the NPAPF NPs showed a continuous increase in size when exposed to solutions with increasing pH values. The investigators attributed this phenomenon to the destruction of the surface charge on the NPs by the zwitterions in the solutions.

Exposure of KB cells to NPAPF NPs at concentrations ranging from 2.5 μM to 20 μM showed that >85% of the cells remained viable even after 48 h as determined with an MTT (3-(4, 5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) cell proliferation assay, indicating that the NPs were nontoxic to the cells (2).

In another study, NPAPF-PEG NPs were shown to have superior photostability compared with the fluorescein isothiocyanate, a NIRF dye that is commonly used for bioimaging (2).

The NPAPF-PEG NPs and NPAPF-PEG-FA NPs were evaluated for the *in vitro* targeting of KB cells exhibiting a high expression of the folate receptor (FR; generated by growing the cells in FA-free growth medium; FA+ cells) or low levels of the receptor (produced by culturing the cells in normal growth medium; FA− cells) (2). For this, the two cell types were exposed to 1 μM of each type of the NPs for 2 h at 37°C and viewed under a confocal laser-scanning fluorescence microscope. Only the FA+ cells were shown to bind the NPAPF-PEG-FA NPs and to generate an intense fluorescence signal. Exposure of the FA+ cells to excess FA (concentration not mentioned) prior to the addition of NPAPF-PEG-FA NPs was reported to reduce the intensity of the fluorescence signal from the cells, indicating that the NPAPF-PEG-FA NPs specifically targeted the FA receptor in the FA+ cells. From this study, the investigators concluded that the NPAPF-PEG NPs can be modified to target cellular components for the detection and visualization of specific cell types (2) because the Fr is usually overexpressed in tumor cells and activated macrophages, but is expressed at low levels in healthy tissues (5).

Animal Studies

Rodents

[PubMed]

The biodistribution of NPAPF-PEG NPs was investigated in Balb/c mice bearing A549 cell tumors (2). The animals (n = 3 mice/time point) were intravenously injected with 93 μM NPAPF-PEG NPs and euthanized at different time points ranging from 1 h postinjection (p.i.) to 48 h p.i., and fluorescence images of the organs of interest were acquired. The fluorescence intensity of each organ was calculated from the images to obtain a semi-quantitative biodistribution of the NPs, and the results were presented as percent of injected dose per gram tissue (% ID/g). At 1 h p.i., the accumulation of NPs in the liver, tumor, intestines, stomach, and kidney was 17.4% ID/g, 17.2% ID/g, 16% ID/g, 13% ID/g, and 8% ID/g, respectively. The accumulation of NPs in all other organs was reported to be <7% ID/g. At 12 h p.i., the uptake of NPs in the liver and the tumor was 25.2% ID/g and 29.5% ID/g NPs, respectively, and all other organs showed an accumulation varying from ~2% ID/g (heart) to ~10% ID/g (intestines). The blood circulation half-life of the NPAPF-PEG NPs was determined to be ~3 h. From this study, the investigators concluded that the uptake of NPAPF-PEG NPs in the cancerous lesions was due to the enhanced permeability and retention (EPR) effect in the tumor blood vessels (2). (For information regarding the EPR effect see Fang et al. (6).)

For whole-body imaging of the animals (number of animals not reported), the mice were injected with NPAPF-PEG NPs as before, and fluorescence images of the rodents were acquired at 12 h p.i (2). Only the tumors were reported to be visible in the animals at this time point.

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.

References

1. Luo S., Zhang E., Su Y., Cheng T., Shi C. *A review of NIR dyes in cancer targeting and imaging.* Biomaterials. 2011;32(29):7127–38. PubMed PMID: 21724249.
2. Yang Y., An F., Liu Z., Zhang X., Zhou M., Li W., Hao X., Lee C.S. *Ultrabright and ultrastable near-infrared dye nanoparticles for in vitro and in vivo bioimaging.* Biomaterials. 2012;33(31):7803–9. PubMed PMID: 22819497.
3. Hong Y., Lam J.W., Tang B.Z. *Aggregation-induced emission: phenomenon, mechanism and applications.* Chem Commun (Camb). 2009;(29):4332–53. PubMed PMID: 19597589.
4. Merian J., Gravier J., Navarro F., Texier I. *Fluorescent nanoprobes dedicated to in vivo imaging: from preclinical validations to clinical translation.* Molecules. 2012;17(5): 5564–91. PubMed PMID: 22576228.
5. Muller C., Vlahov I.R., Santhapuram H.K., Leamon C.P., Schibli R. *Tumor targeting using 67Ga-DOTA-Bz-folate--investigations of methods to improve the tissue distribution of radiofolates.* Nucl Med Biol. 2011;38(5):715–23. PubMed PMID: 21718947.
6. Fang J., Nakamura H., Maeda H. *The EPR effect: Unique features of tumor blood vessels for drug delivery, factors involved, and limitations and augmentation of the effect.* Adv Drug Deliv Rev. 2011;63(3):136–51. PubMed PMID: 20441782.