Indocyanine green–doped calcium phosphate nanoparticles

ICG-CPNPs

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Chemical name:	Indocyanine green–doped calcium phosphate nanoparticles	
Abbreviated name:	ICG-CPNPs	
Synonym:		
Agent category:	Compound	
Target:	Enhanced retention and permeability (EPR)	
Target category:	Non-targeted	
Method of detection:	Optical, near-infrared fluorescence (NIR) imaging	
Source of signal:	Indocyanine green (ICG)	
Activation:	No	
Studies:	• *In vitro* • Rodents	No structure is available in PubChem.

Background

[PubMed]

Optical fluorescence imaging is increasingly being used to monitor biological functions of specific targets in small animals (1-3). However, the intrinsic fluorescence of biomolecules 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 natural background fluorescence interference of biomolecules, providing a high contrast between target and background tissues in small animals. NIR fluorophores have a wider dynamic range and minimal background fluorescence as a result of reduced scattering compared with visible

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fluorescence detection. NIR fluorophores also have high sensitivity, attributable to low background fluorescence, 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 a non-invasive complement to radionuclide imaging in small animals.

Among the various NIR agents, indocyanine green (ICG), with absorption at 780 nm and emission at 820 nm, is the only agent approved by the United States Food and Drug Administration for clinical applications in angiography, blood flow evaluation, and liver function assessment (4). It is also under evaluation in several clinical trials for other applications. However, ICG has a plasma half-life of 2–4 min because it aggregates in the blood and is rapidly cleared from blood circulation (5). ICG is prone to photobleaching and nonspecific quenching *via* its extensive binding to proteins. Calcium phosphate (CP) is present at millimolar concentrations in the blood. CP nanoparticles (CPNPs) have been used to encapsulate organic molecules for intracellular imaging and drug delivery (6). Altinoglu et al. (7) used CPNPs doped with ICG to improve the bioavailability of ICG as a NIR imaging agent in small animals.

Related Resource Links:

- Chapters in MICAD
- Clinical trials (Indocyanine green)
- Drug information in FDA

Synthesis

[PubMed]

Two reverse microemulsions were formed by a cyclohexane/nonylphenoxyl (glycolether) (Igepal CO-520)/water system $CaCl_2$ (0.065 mM) was added to 14 mL of a 29 vol % solution of Igepal CO-520 in cyclohexane to form microemulsion A (7). Microemulsion B was produced by adding disodium phosphate (0.39 mM) and disodium silicate (0.54 mM) to an identical Igepal CO-520/cyclohexane mixture. ICG (5.2 mM) was added to microemulsion B. The microemulsions were equilibrated under constant stirring for 1 h before being combined to form a microemulsion mixture (microemulsion C). Microemulsion C was stirred for 2 min before the dispersant, 0.225 mM sodium citrate, was added, and the solution was stirred for an additional 15 min. The product, ICG-CPNPs, was isolated with column chromatography. The size of ICG-CPNPs was 16 ± 0.23 nm as measured with transmission electron microscopy.

To prepare polyethylene glycol–conjugated ICG-CPNPs (PEG-ICG-CPNPs), a 4-mL aliquot of the carboxylateCPNPs (5×10^{13} particles) was chemically conjugated with methoxypolyethylene glycol amine (mPEG-amine) through an ethyl-*N*-(3-dimethylaminopropyl)-*N*′-hydrochloride carbodiimide (EDAC) reaction. EDAC (1 mg) and mPEG-amine (10 mg) were added to the sample under continuous stirring (six-fold excess for monolayer surface coverage). The particles were reacted for 15 h at 50°C to

form amide linkages between the carboxylate surfaces and the mPEG-amine. The mixture was then filtered through a centrifuge filter to remove excess EDAC and unreacted mPEG-amine.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

TEM images showed that the doped ICG was uniformly distributed in the CP matrix of each NP (7). The concentration of ICG was estimated by fluorescence emission intensity to be 0.01 mM in 10^{13} NPs/mL. The fluorescence emission intensity of each ICG-CPNP was 1,000 times that of one ICG molecule because it contains more than 1 ICG. The quantum efficiency of ICG-CPNPs (0.049 ± 0.003) was one-fold greater than that of free ICG (0.028 ± 0.001) in saline buffer. The emission spectra and photostability for ICG-CPNPs were more stable than those of free ICG in various media. *Ex situ* imaging studies were performed with porcine muscle tissue. ICG and ICG-CPNPs solutions (0.1 mM) were injected at different depths in the porcine muscle tissue. Detectable fluorescence signal was still observed at 3 cm for ICG-CPNPs as compared with 1.5 cm for free ICG.

Animal Studies

Rodents

[PubMed]

Altinoglu et al. (7) performed *in vivo* whole-body NIR fluorescence imaging studies of PEG-ICG-CPNPs and ICG (200 nmol/mouse) in nude mice (n = 4/group) bearing human MDA-MB-231 breast adenocarcinoma tumors. Images were obtained at 3, 24, and 96 h after intravenous injection. PEG-ICG-CPNPs exhibited clear signal intensity at 24 h after injection. The tumors were still clearly visualized at 96 h. High fluorescence intensities were observed *ex vivo* in the liver and kidneys at 10 min after injection. On the other hand, no detectable signal was observed in the tumors with ICG at 24 h and 96 h. The authors suggested that PEG-ICG-CPNPs provide prolonged circulation times with passive tumor accumulation at 24 h and 96 h after injection.

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.

References

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