Quantum dots-deoxycholic acid (DOCA)-conjugated low molecular weight heparin (LMWH) nanoparticles

Q-LHD

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Chemical name:	Quantum dots-deoxycholic acid (DOCA)-conjugated low molecular weight heparin (LMWH) nanoparticles	
Abbreviated name:	Q-LHD, Q-LHD5	
Synonym:		
Agent category:	Compound	
Target:	Bile acid transporter	
Target category:	Transporter	
Method of detection:	Optical, near-infrared fluorescence (NIR)	
Source of signal:	Quantum dot (QD)	
Activation:	No	
Studies:	• *In vitro* • Rodents	Structure is not available in PubChem.

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 poses a problem when fluorophores that absorb visible light (350–650 nm) are used. Near-infrared (NIR) fluorescence (650–1,000 nm) detection avoids the background

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fluorescence interference of natural biomolecules, providing a high contrast between target and background tissues. NIR fluorophores have 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 complement to radionuclide imaging in small animals (4, 5).

Fluorescent semiconductor quantum dots (QDs) are nanocrystals made of CdSe/CdTe/ZnS with radii of 1–10 nm (6-8). They can be tuned to emit fluorescence signals in a range of wavelengths by changing their sizes and composition, thus providing broad excitation profiles and high absorption coefficients. They have narrow, symmetric emission spectra with long, excited-state lifetimes (20–50 ns) compared to those of fluorescent dyes (1–10 ns). They possess good quantum yields of 40%–90% and high extinction coefficients. QDs are more photo-stable than conventional organic dyes. They can be coated and capped with hydrophilic materials for additional conjugation with biomolecules, such as peptides, antibodies, nucleic acids, and small organic compounds, which have been tested *in vitro* and *in vivo* (8-12). Although many cells have been labeled with QDs *in vitro* with little cytotoxicity, there are limited studies of long-term toxicity of QDs in small animals (13-21). Little is known about the toxicity and the mechanisms of clearance and metabolism of QDs in humans.

Low molecular weight heparin (LMWH) is a glycosaminoglycan that is widely used as an anticoagulant agent (22). LMWH was reported to inhibit cancer progression through its anti-angiogenic and anti-metastatic effects (23). However, LMWH has to be injected intravenously due to its poor absorption in the intestines. Deoxycholic acid (DOCA)-conjugated LMWH (LHD) was found to be absorbed in the small intestine *via* the bile acid transporter after oral administration to rodents and monkeys (24). Khatun et al. (25, 26) prepared LHD micelles loaded with NIR fluorescence QDs (Q-LHD nanoparticles) as an oral imaging agent for optical imaging of the small intestines.

Related Resource Links:

- Chapters in MICAD (QDs, heparin)
- Gene information in NCBI (bile acid transporter)
- Articles in Online Mendelian Inheritance in Man (OMIM) (bile acid transporter)
- Clinical trials (heparin)
- Drug information in FDA (heparin)

Synthesis

[PubMed]

LHD was prepared by conjugation of the hydroxyl group of DOCA with the carboxylic group of LMWH (26). DOCA was aminated by reaction with 4-methylmorpholine and

ethylenediamine overnight at room temperature. An aqueous solution of LMWH (pH 5) was added to a solution of the aminated DOCA, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide and *N*-hydroxysuccinimide. After incubation for 30 min, LHD (diameter = 187 ± 32 nm) was purified with dialysis, with 44% yield and 82% purity. There were five DOCA moieties per LMWH. QDs (CdSe/CdTe) (56 nmol Cd, 7 nm in diameter) with an emission maximum at 675 nm were mixed with 50 mg LHD in chloroform for 18 h. After evaporation of the organic solvent, the mixture was dialyzed for 48 h with an aqueous solution. Q-LHD nanoparticles were isolated with filtration, with 81% yield. Q-LHD nanoparticles had a diameter of 220 ± 26 nm as measured with electrophoretic light scattering (ELS). The zeta potential values were −68, 25, and −7.3 mV for LHD, QD, and Q-LHD, respectively. The diameters of LHD and Q-LHD nanoparticles remained similar after incubation in phosphate-buffered saline (PBS) for 2 h at pH 5, 7, and 9 as measured with ELS. The diameter and fluorescence intensity of Q-LHD remained little changed after 5 d incubation in 10% fetal bovine serum.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Khatun et al. (26) showed that there was little release of QDs from Q-LHD for up to 5 d (room temperature) at pH 7.4 and pH 9. On the other hand, the release of QDs was higher at pH 1.5 and pH 5 compared with pH 7.4 and pH 9. *In vitro* cytotoxicity and cellular uptake assays were performed by incubation of Caco-2 cells with different concentrations of Q-LHD (0.1–100 μg/mL) for 24 h and 48 h at 37°C. Cell viability was 80%–95% at 24 h and 60%–70% at 48 h. Confocal laser scanning microscopy showed the presence of Q-LHD on the cell surface and inside the cell membrane after 1 h at 37°C.

Animal Studies

Rodents

[PubMed]

Khatun et al. (26) performed NIR fluorescence imaging studies with female nude mice (*n* = 4/group) at 2–36 h after oral administration of 2.5 mg/kg and 5.0 mg/kg Q-LHD. Whole-body small animal images were obtained with a time-domain diffuse optical tomography system. The QD signal was found mainly in the stomach at 2 h and was detected in the duodenum, jejunum, and ileum at 8 h. The QD signals were clearly visualized in the lung, heart, liver, spleen, rectum, kidneys, small intestines, and stomach at 8 h. By 24 h, most of the signal intensity from the 2.5 mg/kg dose in the stomach was gone, but the signal from the 5.0 mg/kg dose persisted at this time point. By 36 h, most of the signal intensity was gone from the images. Analysis of the excised tissues at 36 h showed only a negligible amount of Cd^{3+} (<0.0045 μg Cd/g tissue) in the kidney, liver, lung, heart, duodenum, jejunum, and ileum. Oral administration of taurocholic acid (TCA) (0, 25, and 50 mg/kg) 1.5 h before oral administration of 2.5 mg/kg Q-LHD

exhibited a dose-dependent inhibition of signal intensity in the small intestines at 8 h. The highest dose of TCA completely inhibited the signal intensity in the intestinal area, suggesting that TCA and DOCA bind to the bile acid transporter in the small intestines.

Khatun et al. (25) performed *ex vivo* NIR fluorescence imaging studies with female nude mice (n = 3/group) at 8 h after oral administration of 2.5 mg/kg or 5.0 mg/kg Q-LHD. The highest fluorescence intensity was observed in the ileum and was expressed as 100% relative fluorescence units (RFU) with the higher oral dose. The jejunum showed 75% RFU, whereas the duodenum, stomach, liver, heart, and kidney showed <10% RFU. The oral dose of 2.5 mg/kg showed slightly lower RFU than the 5.0 mg/kg dose in the tissues examined. Plasma concentration of Q-LHD was assessed with anti-Factor Xa activity of LMWH. Plasma concentration of oral doses of Q-LHD appeared at 30 min and peaked at 120 min before declining. The maximum plasma concentrations were 0.82 ± 0.19 and 0.95 ± 0.30 IU/mL for 2.5 mg/kg and 5.0 mg/kg Q-LHD, respectively. NIR fluorescence measurements of the plasma followed the time pattern for anti-Factor Xa measurements. Therefore, Q-LHD nanoparticles were rapidly absorbed in the intestines and found in the blood circulation. Transition electron microscopy showed the presence of Q-LHD nanoparticles in the jejunum and ileum, but not in the stomach and duodenum, at 8 h.

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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