IRDye 800CW-Human serum albumin

HSA800

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Chemical name:	IRDye 800CW-Human serum albumin	
Abbreviated name:	HSA800	
Synonym:		
Agent category:	Polypeptide	
Target:	Non-targeted	
Target category:	Non-targeted, lymph node trapping	
Method of detection:	Optical, near-infrared (NIR) fluorescence imaging	
Source of signal:	IRDye 800CW	
Activation:	No	
Studies:	• Rodents • Non-primate non-rodent mammals • Humans	Click on protein, nucleotide (RefSeq), and gene for more information about HSA.

Background

[PubMed]

Optical fluorescence imaging is increasingly being used to monitor biological functions of specific targets (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 fluorescence detection. NIR fluorophores also have high sensitivity, attributable to low

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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 alternative to radionuclide imaging in small animals or with probes in close proximity of the target in humans (4, 5). Among the various optical imaging agents, only indocyanine green (ICG), with NIR fluorescence absorption at 780 nm and emission at 820 nm, is approved by the United States Food and Drug Administration for clinical applications in angiography, blood flow evaluation, and liver function assessment. It is also under evaluation in several clinical trials for other applications, such as optical imaging and mapping of both the lymphatic vessels and lymph nodes in cancer patients for surgical dissection of tumor cells and endoscopic imaging of the pancreas and colon.

The sentinel lymph node (SLN) is considered to be the first lymph node to receive lymphatic flow from tumor sites and therefore, will contain metastatic tumor cells (6). SLN mapping has been used in diagnosis of metastasis of solid tumors (7). Radical lymph node dissection is performed in patients with malignant cells in the SLNs. Presently, SLN mapping is performed by a combination of radioactive tracer and blue dye, which require a radiologist. The current procedure is also time-consuming and requiring a steep learning curve. NIR quantum dots (QDs) has been studied in SLN mapping in small animals (4, 8). However, there are only limited studies of long-term toxicity of QDs in animals (9). IRDye CW800-CW is an indocyanine-type NIR fluorophore with peak absorption at 775 nm, and peak excitation emission at 796 nm. It provides a quantum yield of 9% with an extinction coefficient of 242,000 $M^{-1}cm^{-1}$. It has a molecular weight of 962 Da. Human serum albumin (HSA) has been successfully conjugated with IRDye 800CW to form IRDye 800CW-HSA (HSA800) for non-invasive NIR mapping of SLNs in small animals (10-12).

Related Resource Links:

- Chapters in MICAD (HSA)
- Gene information in NCBI (HSA)
- Articles in Online Mendelian Inheritance in Man (OMIM) (HSA)
- Clinical trials (ICG)
- Drug information in FDA (ICG)

Synthesis

[PubMed]

Commercially available IRDye 800CW-*N*-hydroxysuccinimide ester (LI-COR, Lincoln, NE) was used to conjugate HSA to form HSA800, which was purified by gel filtration chromatography to a purity of >98% (10). The molar ratio of IRDye 800CW to HSA was estimated to be 3.0 by spectrometric measurements. The hydrodynamic diameter of HSA800 was 7.4 nm with a molecular weight of 70 kDa. HSA800 has an extinction of coefficient of 237,000 $M^{-1}cm^{-1}$ and a quantum yield of 0.12 with a maximal excitation

wavelength of 784 nm and a maximal emission wavelength of 802 nm in fetal bovine serum.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

No publication is currently available.

Animal Studies

Rodents

[PubMed]

Parungo et al. (11) compared the accumulation of HSA800 (7.4 nm in diameter, 70 kDa) and QDs (15-20 nm, 400-800 kDa) in rats (n = 4) using an intraoperative NIR fluorescence imaging system. HSA800 (12 nmol/kg) and QDs (6.8 nmol/kg) injected into the peritoneal space of rats and fluorescence signals were monitored at 10-20 min, and 1, 4, and 24 h after injection. QDs drained to the celiac, superior mesenteric, and periportal lymph node groups within 20 min. This pattern remained the same at the later time points. HSA800 drained to these same lymph node groups at early time points but continued flowing to the mediastinal lymph nodes via the thoracic duct by I h. After bowel resection, both tracers were found in the thoracic, not abdominal lymph node groups. Additionally, HSA800 was no longer found in the thoracic duct but in the anterior chest wall and diaphragmatic lymphatics.

Other Non-Primate Mammals

[PubMed]

Ohnisbi et al. (10) performed SLN mapping of the intestine of pigs by injections with indocyanine green (ICG, 1.2 nm in diameter, 1 nmol), CW800-CA (1.3 nm, 1 nmol), ICG:HSA (7.3 nm, 1 nmol), HSA800 (7.4 nm, 1 nmol), QDs (15-20 nm, 0.04 nmol), and nanocoll800 (50 nm, 0.4 nmol). The best SLN retention was nanocoll800, followed by HSA800, ICG:HSA, and QDs. ICG and CW800-CA were not retained by the SLNs at all. A more rigorous evaluation of contrast agents was performed using the porcine lower limb by subcutaneous injections. HSA800 revealed ultra-fine lymphatic channels flowing to a single SLN. ICG failed to identify the SLN, whereas ICG:HSA showed barely the lymphatics, but not the SLNs.

Tanaka et al. (12) performed SLN mapping in Sinclair swine with spontaneous melanoma metastatic to regional lymph nodes. ICG:HSA (1 nmol), HSA800 (1 nmol) and QDs (0.01 nmol) were tested in six pigs with 4 separate primary melanomas by peri-tumoral, subcutaneous injections. All 12 injections identified the associated SLNs (proximal and distal) in the whole animal imaging. ICG:HSA exhibited slightly lower signal intensity

than HSA800 and QDs. Pathological analysis confirmed the presence of metastatic tumor cells in proximal and distal SLNs but not in the nearby control lymph nodes.

Hutteman et al. (13) identified 32 of 32 (100%) SLNs in the colon and rectum of pigs (n = 4) under both *in vivo* and *ex vivo* conditions after injection of HSA800 (1 nmol).

Non-Human Primates

[PubMed]

No publication is currently available.

Human Studies

[PubMed]

Hutteman et al. (13) injected HSA800 (50 nmol) submucosally circumferentially with a 5-mm margin around the tumors in 24 colorectal cancer patients. SLN mappings were performed in *ex vivo* tissue specimens. SLNs were identified in all 24 patients within 5 min after injection of HSA800 with nine patients showing lymph node metastases. In one patient out of 24 patients, a 3-mm mesenteric metastasis was found adjacent to a tumor-negative SLN (false-negative).

References

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