5-Carboxy-fluorescein-labeled octreotate

OcF

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Created: July 27, 2010; Updated: August 16, 2010.

Chemical name:	5-Carboxy-fluorescein-labeled octreotate	
Abbreviated name:	OcF	
Synonym:		
Agent Category:	Polypeptides	
Target:	Somatostatin receptors (SSTRs)	
Target Category:	Receptors	
Method of detection:	Optical imaging (miniaturized confocal laser-scanning fluorescence microscopy)	
Source of signal / contrast:	5-Carboxy-fluorescein	
Activation:	No	
Studies:	• *In vitro* • Rodents	No structure is available.

Background

[PubMed]

Octreotate labeled with 5-carboxy-fluorescein, abbreviated as OcF, is an optical imaging agent developed by Fottner et al. for in vivo imaging of somatostatin receptors (SSTRs) in neuroendocrine tumors with miniaturized confocal laser-scanning microscopy (CLM) (1). SSTRs are highly expressed in neuroendocrine tumors (2, 3). The excitation and emission wavelengths of 5-carboxy-fluorescein are 492 nm and 518 nm, respectively. The molecular weight of octreotate is 1033.3.

CLM is an emerging technique that integrates the miniaturized components of a confocal laser scanner into the tip of a conventional endoscope (4-7). During imaging, a solid laser

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NLM Citation: Shan L. 5-Carboxy-fluorescein-labeled octreotate. 2010 Jul 27 [Updated 2010 Aug 16]. In: Molecular Imaging and Contrast Agent Database (MICAD) [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2004-2013.

delivers an excitation wavelength of 488 nm on the surface of target organs, and light emission is detected at 505–585 nm. Serial images are collected at a scan rate of 0.8 frames/s at 1024 × 1024 pixels or 1.6 frames/s at 1024 × 512 pixels. The laser power output can be adjusted from 0 μW to 1000 μW to achieve appropriate tissue contrast. The optical slice thickness is usually 7 μm, with a lateral resolution of 0.7 μm and an imaging plane depth to 250 μm from the tissue surface. Tissue images are produced on the basis of different refractive indices from biological tissues, and image contrast is enhanced with application of contrast agents such as intravenous sodium fluorescein or topical acriflavine (7, 8). Virtual histology can be created with CLM, which allows visualization of pathological changes at cellular and subcellular levels in addition to standard videoendoscopy (5, 7). Studies with CLM have demonstrated its effectiveness in screening inflammatory and early malignant diseases of the gastrointestinal tract (1). However, the CLM technique does not allow clear, simultaneous, in vivo imaging of the tissue anatomy together with a dynamic visualization of molecular targets like the SSTRs (1).

Fottner et al. developed a miniaturized CLM technique that allows the specific molecular imaging of SSTRs in vivo and in real time (1). For selective visualization of SSTRs, a contrast agent for CLM was specifically synthesized by conjugating 5-carboxy-fluorescein to octreotate (referred to as OcF). With the miniaturized CLM and OcF, equal morphological resolution of SSTR-expressing cell structures (pancreatic islet cells, renal tubular cells, and neuroendocrine tumors) was achieved, unlike with standard histological tissue investigation. Fottner et al. concluded that the CLM technique together with OcF allows specific visualization of SSTR-expressing neuroendocrine tumor cells, while expression of SSTRs and SSTR-mediated endocytosis of somatostatin and its analogs in neuroendocrine tumors represent the molecular basis for various clinical diagnostic and therapeutic applications (1).

Related Resource Links:

- MICAD chapters on SSTR imaging
- Gene information on SSTRs
- Articles of SSTRs in OMIM
- Somatostatin analogs in PubChem
- Bioassays of SSTRs in PubChem
- SSTR-related clinical trials in ClinicalTrial.gov

Synthesis

[PubMed]

Fottner et al. synthesized OcF with Fmoc solid-phase peptide synthesis and labeled the peptide with 5-carboxy-fluorescein on solid phase (1). The labeled product (OcF) was cleaved from resin and purified. The purity of OcF was >99%. The labeling efficiency and the number of fluorescein molecules per octreotate were not reported.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

The affinity of OcF to SSTRs was tested against ^{125}I-Tyr3–labeled octreotide with a membrane preparation from rat cerebral cortex (predominantly expressing SSTR subtype 2 (SSTR2)) (1). The 50% inhibitory concentration (IC_{50}) of OcF was 6.2 nmol, which was comparable to the IC_{50} values of [^{111}In-DTPA]octreotide and [^{90}Y-DOTA-Tyr]octreotide (22 nmol and 11 nmol, respectively). [^{111}In-DTPA]octreotide and [^{90}Y-DOTA-Tyr]octreotide are currently used for clinical imaging purposes (2, 3, 9).

The biological activity of OcF was evaluated after incubation with AR42J and Panc1 cells (1). AR42J is a rat pancreatic cancer cell line with abundant expression of SSTR2–SSTR5, with SSTR2 being the predominant subtype. Panc1 is a human pancreatic adenocarcinoma cell line with barely detectable expression of SSTRs. Unlabeled octreotide resulted in a >50% suppression of the AR42J cell proliferation at 5 µmol, whereas OcF had similar activity only at >100 µmol. However, both agents had a similar maximum antiproliferative effect at 250 µmol. The results suggested that OcF had a comparable maximal antiproliferative effect at higher but still physiological concentrations. In SSTR-negative Panc1 cells, none of the unlabeled octreotide and OcF had any antiproliferative effect.

In vitro SSTR imaging was performed with conventional fluorescence and confocal microscopy (1). Time- and dose-dependent staining of the AR42J cells was observed with a maximum intensity at 100 µmol and a still detectable contrast at 0.1 µmol OcF. Best contrast was achieved at a concentration of 10 µmol OcF after incubation for 30 min. Similar results were obtained when the miniaturized CLM was applied. Cell staining with OcF was blocked when the cells were pre-incubated with unlabeled octreotide. When SSTR-negative Panc1 tumor cells were used, no fluorescence was observed after incubation with OcF.

Animal Studies

Rodents

[PubMed]

In vivo imaging of SSTR-expressing pancreatic islet cells, renal tubular cells, and tumor xenografts was performed with CLM after intravenous injection of OcF (1). The mouse pancreas and kidneys were surgically exposed, and the miniaturized CLM probe was directly placed onto the tissues.

Pancreas imaging showed that a subpopulation of cells started to exhibit contrast at the cell surface 15 min after OcF injection. Internalization and cytoplasmic distribution were observed after an additional 15–30 min. The bright cells that were clearly contrasted with

surrounding tissue represented the SSTR-positive islet cells as confirmed with ex vivo immunohistochemistry (1).

Kidney imaging showed that the tubular cells of the cortex already had weak superficial staining a few minutes after injection, and at 15–30 min had a bright surface staining and the beginning of cytoplasmic distribution. Maximum staining intensity was reached after 30–45 min and gradually attenuated after 60 min, with a bright-contrast enhancement of the renal tubular system after tubular secretion. Not all tubular cells of the renal cortex were contrasted after OcF application, which was in good accordance with ex vivo immunohistochemistry that showed an irregular expression of the SSTR2 in renal tubular cells (1).

In tumor xenograft imaging, SSTR-expressing AR42J tumor cells displayed a bright cell surface and cytoplasmic staining (1). SSTR-Negative Panc1 tumor cells were detectable only by the background fluorescence with twice the amount of OcF. The sensitivity and specificity of the miniaturized CLM with OcF for detection of SSTR-positive cells in vivo were 87.5% and 100%, respectively.

Other Non-Primate Mammals

[PubMed]

No references are currently available.

Non-Human Primates

[PubMed]

No references are currently available.

Human Studies

[PubMed]

No references are currently available.

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