^{64}Cu-1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid-quantum dot-vascular endothelial growth factor

^{64}Cu-DOTA-QD-VEGF

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Created: July 1, 2008; Updated: August 12, 2008.

Chemical name:	^{64}Cu-1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid-quantum dot-vascular endothelial growth factor	
Abbreviated name:	^{64}Cu-DOTA-QD-VEGF, ^{64}Cu-DOTA-QD-VEGF$_{121}$	
Synonym:		
Agent Category:	Peptide	
Target:	Vascular endothelial growth factor receptor 2 (VEGFR-2)	
Target Category:	Receptor binding	
Method of detection:	Positron emission tomography (PET), near-infrared (NIFR) optical imaging	
Source of signal/ contrast:	^{64}Cu, quantum dot (QD705)	
Activation:	No	
Studies:	• *In vitro* • Rodents	No information is currently available in PubChem.

Background

[PubMed]

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NLM Citation: Zhang H. ^{64}Cu-1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid-quantum dot-vascular endothelial growth factor . 2008 Jul 1 [Updated 2008 Aug 12]. In: Molecular Imaging and Contrast Agent Database (MICAD) [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2004-2013.

Vascular endothelial growth factor (VEGF), also known as vascular permeability factor, is a homodimeric glycoprotein weighing ~45 kDa (1). The VEGF family consists of six groups: VEGF-A, -B, -C, -D, -E, and the placental growth factor (PIGF) (2). Structurally, VEGFs are related to the platelet-derived growth factors (PDGF), and they all contain the characteristic eight-cysteine residues known as the cysteine knot motif (3). Intrachain and interchain disulfide bonds are formed between these cysteine residues in conserved positions (2). VEGFs bind specifically to three cell-surface receptor tyrosine kinases, including fms-like tyrosine kinase-1 (Flt-1) or VEGF receptor-1 (VEGFR-1), kinase insert domain-containing receptor (KDR) or VEGRF-2, and Flt-4 or VEGFR-3. Each VEGFR contains a 750-amino acid-residue extracellular domain that is organized into seven immunoglobulin-like folds. VEGF and VEGFRs have been implicated in angiogenesis in many solid tumors, including breast cancer, colon cancer, hepatoma, bladder cancer, gastric cancer, and prostate cancer (3). VEGFR-2 (~220 kDa) is expressed exclusively in endothelial cells in cell differentiation, tumor vascularization, and metastasis. VEGF-A (the original VEGF) binds to the second and third extracellular immunoglobulin G loop of VEGFR-2 with a dissociation constant of ~100 pM (4). Hypoxia appears to be an important stimulus for producing VEGF in malignant and normal endothelial cells (3). Upon binding to its receptor VEGFR-2, VEGF-A elicits a pronounced angiogenic response, so it is considered as a predominant stimulator of angiogenesis. Human VEGF-A has five different isoforms generated by alternative slicing of a single pre-mRNA species, VEGF-A_{121}, VEGF-A_{145}, VEGF-A_{165}, VEGF-A_{189}, and VEGF-A_{206}, which comprises 121,145,165, 189, and 206 amino acids, respectively (2). These isoforms differ in their ability to bind to heparin sulfate and extracellular matrix (ECM).

Quantum dots (QDs) are semiconductor nanocrystals of 2 to 10 nm in diameter (200–10,000 atoms) that possess a quantum confinement effect (hence the name "quantum dots") caused by the restriction of electrons and holes in all three dimensions (5, 6). Like classic semiconductors that are composed of two types of atoms from the II/VI or III/V group elements in the periodic table, the nanocrystals have a valence band and a conduction band separated by an energy gap (band gap). Upon excitation, an electron is promoted from the filled valence band to the largely empty conduction band, which creates a positive vacancy "hole" in the valence band. The spatial separation (Bohr radius) of this electron-hole pair ("exciton") is typically 1 to 10 nm for most semiconductors (6). The quantum confinement arises when one of the dimensions in the nanocrystals becomes comparable to its Bohr radius, these valence/conduction bands are quantized with an energy value that is directly related to the nanocrystal size. Thus, the excitons are confined in a manner similar to a particle-in-the-box problem, leading to a finite band gap and discretization of energy levels. When the electron fills the vacancy in the valence band, light of a certain wavelength is emitted, which corresponds to the respective band gap energy that is a function of nanocrystal size. For instance, the emission wavelength is 550 nm for 3-nm CdSe QDs and 650 nm for 7-nm CdSe QDs (7). The wavelength is also a function of semiconductor compositions, i.e., 5-nm CdTe has an emission wavelength of 700 nm, which is much higher than the 620 nm for 5-nm CdSe (8). QDs are 100 to 1,000 times more stable against photobleaching and are 10 to 100 times brighter than organic

dyes. QDs have relatively long fluorescence lifetime (20–50 ns), which allows for time-resolved detection of their emitted fluorescence. For biological applications, QDs are generally encapsulated with biocompatible polymers that can increase their hydrodynamic diameter as much as two-fold (9). When their size is <5 nm, QDs are quickly cleared by renal filtration, whereas larger particles are more likely to be taken up by the reticuloendothelial system before reaching the targeted disease sites. Thus, after systematic administration, non-targeted QDs and some targeted QDs accumulate in substantial quantities in reticuloendothelial system, including the phagocytic cells in the liver, spleen, lymph nodes, and bone marrow (5). QDs can also accumulate in solid tumor tissue through the enhanced permeability and retention (EPR) effect regardless of whether they are conjugated with targeting ligands. As a whole, QDs have been widely used in cell trafficking, vasculature imaging, sentinel lymph node mapping, neural imaging, and targeting imaging (5).

^{64}Cu-1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid-QD-VEGF factor (^{64}Cu-DOTA-QD-VEGF) is a multimodal agent used for imaging VEGFRs with positron emission tomography (PET) and near-infrared (NIFR) optical imaging (10). ^{64}Cu-DOTA-QD-VEGF consists of three components. An amine-functionalized QD (QD705) is used as a NIFR sensor and as a platform for carrying target-specific ligands. VEGF proteins are attached to the surface of QDs for recognition of VEGFRs. Complexes of the macrocyclic chelating agent DOTA with ^{64}Cu(II) (^{64}Cu-DOTA) are also covalently attached to the surface of QDs. ^{64}Cu is a positron-emitting radionuclide with an intermediate half-life (12.7 h) that decays by positron (β^+) with a branching factor of 17.4% and a maximum β^+ energy of 0.653 MeV (11). ^{64}Cu has been used as a radiotracer in PET imaging and as a radiotherapy agent in cancer treatment. QD705, which is commercially available, comprises a CdTe core shell with a thin layer of ZnS and polyethylene glycol (PEG2000)-attached amine groups (12). The emission wavelength of QD705 (705 nm) is located in the NIRF region (700–900 nm) where the absorbance of all biomolecules reaches a minimum (10). The use of a ZnS shell can increase the quantum yield of CdTe up to 30% to 50% (5). The PEG is used to decrease surface charge, increase colloidal stability of QDs, and reduce non-specific binding of QDs (13). Although QDs are used to examine cellular alteration, their *in vivo* detection is limited by the penetration depth of light. Thus, PET as a highly sensitive and quantitative modality can provide complementary information about tissues in depth. This PET/NIRF dual-modality probe may combine the advantages of QD optical imaging and PET imaging to assess the pharmacokinetics and targeting efficacy of QDs.

Synthesis

[PubMed]

Chen et al. briefly described the synthesis of ^{64}Cu-DOTA-QD-VEGF (10). Initially, DOTA was activated with 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) and sulfo-*N*-hydroxy-sulfosuccinimide (SNHS) in a molar ratio of 10:5:4 (pH 5.5). The activated DOTA and 4-maleiidobutyric acid *N*-hydroxy-sulfosuccinimide (MAL-NHS)

were added to the solution of QDs (QD705) in a molar ratio of 500:500:1 (pH 8.5) to produce DOTA-QD-MAL. $VEGF_{121}$ was thiolated *in situ* by reaction with *N*-succinimidyl-S-acetylthioacetate (SATA), and then conjugated to DOTA-QD-MAL in a ratio of 10:1 (pH 7.0) to give DOTA-QD-VEGF. Complexation of DOTA-QD-VEGF with ^{64}Cu(II) (pH 6.5) produced a >90% efficiency for ^{64}Cu labeling. The radioactive specificity is 60 pmol QDs per mCi of ^{64}Cu.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Chen et al. examined the *in vitro* binding specificity of ^{64}Cu-DOTA-QD-VEGF to VEGFR-2 (10). Porcine aortic endothelia (PAE/VEGFR-2) cells transfected with human VEGFR-2 were incubated with 1 nM DOTA-QD-VEGF in serum-free F12 Hams growth medium for 30 min and then examined with fluorescence microscope. The QD fluorescence overlaid well with the PAE/VEGFR-2 cells, suggesting a receptor-mediated internalization of the QD conjugate caused by a specific binding of DOTA-QD-VEGF to VEGFR-2. In comparison, no significant staining was observed for DOTA-QD-VEGF that was incubated with VEGFR-negative PAE cells. The binding affinity of DOTA-QD-VEGF was examined in PAE/VEGFR-2 cells in the presence of competitive ligand ^{125}I-VEGF165. Similar to VEGF, the QD conjugate (DOTA-QD-VEGF) inhibited the binding of ^{125}I-VEGF165 to VEGFR-2 in a dose-dependent manner. The 50% inhibition concentration was 4.1 nM for DOTA-QD-VEGF, which was very close to the 5.3 nM for VEGF, indicating that the QD conjugation caused no significant change in its VEGFR-2 binding affinity.

Animal Studies

Rodents

[PubMed]

The uptake of ^{64}Cu-DOTA-QD-VEGF was first examined in tumor-bearing mice *ex vivo* with PET and NIRF imaging (10). Athymic mice were inoculated with U87MG (human glioblastoma) cells on the front flank. When the tumor grew to 200–500 mm^3 (3–4 weeks), the mouse was intravenously injected with ^{64}Cu-DOTA-QD-VEGF (20 pmol QD per 7–14 MBq ^{64}Cu) and euthanized 4 h later. The major organs (liver, spleen, U87MG tumor, heart, lung, kidneys, bone, and muscle) were collected. Half of the tissue was quickly embedded for subsequent fluorescence microscopy. The other harvested tissue was used for microPET and NIRF imaging. For both imaging modalities, all major organs exhibited similar intensity patterns with a strong signal found in liver, spleen, and bone. The U87MG tumors demonstrated a higher uptake than the heart, kidney, and muscle. The QD fluorescence in the tumor tissue exhibited a non-homogeneous distribution. The U87MG tumor tissues were incubated with rat anti-mouse VEGFR-2 antibody and visualized with FITC-conjugated goat anti-rat antibody for immunofluorescence staining.

The results demonstrated that DOTA-QD-VEGF was found co-localized with the VEGF/VEGFR-2 on the tumor vasculature with a small portion of extravasated QD conjugate.

The uptake of DOTA-QD-VEGF was then examined in the same *in vivo* tumor model with NIRF imaging (10). Athymic nude mice bearing subcutaneous U87MG tumors (5-8 nm in diameter) were injected intravenously with DOTA-QD-VEGF (200 pmol QD). Tumor fluorescence intensity increased over time and exhibited good contrast with respect to the background tissue. In comparison, no tumor contrast was observed in mice injected with DOTA-QD as a control. The non-specific uptake of both DOTA-QD and DPTA-QD-VEGF was apparent, as shown in the particularly bright fluorescence found in the bone marrow, spleen, and liver. The uptake of ^{64}Cu-DOTA-QD-VEGF was further examined in the same *in vivo* tumor model with PET imaging (10). Athymic nude mice bearing subcutaneous U87MG tumors were injected intravenously with ^{64}Cu-DOTA-QD-VEGF (20 pmol QD per 7–14 MBq ^{64}Cu). PET images collected at multiple time points after injection demonstrated a prominent uptake of the QD conjugates in the liver, spleen, lymph node, and bone marrow. Comparing the PET images with the NIRF image, the signal in the tumors demonstrated a different pattern: the signal in NIRF decreased with time, whereas the signal in PET increased. This may reflect a receptor-mediated internalization of ^{64}Cu-DOTA-QD-VEGF followed by degradation of the QD conjugate that led to some trapping of ^{64}Cu inside the tumor. As a control, mice were injected with ^{64}Cu-DOTA-QD. The uptake of ^{64}Cu-DOTA-QD-VEGF in the mice with the U87MG tumors was much higher than in the mice injected with ^{64}Cu-DOTA-QD. No significant differences were found in the mouse liver uptake, which were both ~50% injected dose (ID)/g of tissue. The tumor uptake of ^{64}Cu-DOTA-QD-VEGF was 1.5 ± 0.6% ID/g, 2.8 ± 0.3% ID/g, 3.8 ± 0.6% ID/g, and 4.2 ± 0.5% ID/g at 1,4,16, and 24 h. The uptake of ^{64}Cu-DOTA-QD was ≤1% ID/g.

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.

NIH Support

CA 119053, CA 121842, CA 102123, CA 114747, CA 119367, CA 93862

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