^{64}Cu-1,4,7,10-Tetraazacyclodecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid-vascular endothelial growth factor

^{64}Cu-DOTA-VEGF

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Chemical name:	^{64}Cu-1,4,7,10-Tetraazacyclodecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid- vascular endothelial growth factor	
Abbreviated name:	^{64}Cu-DOTA-VEGF	
Synonym:	^{64}Cu-DOTA-VEGF$_{121}$, ^{64}Cu-VEGF, ^{64}Cu-VEGF$_{121}$	
Agent Category:	Peptide	
Target:	Vascular endothelial growth factor receptors (VEGFR or VEGF receptors)	
Target Category:	Receptor binding	
Method of detection:	Positron emission tomography (PET)	
Source of signal:	^{64}Cu	
Activation:	No	
Studies:	• *In vitro* • Rodents • Humans	Click on protein, nucleotide (RefSeq), and gene for more information about VEGF.

Background

[PubMed]

^{64}Cu-1,4,7,10-Tetraazacyclodecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid (DOTA)-vascular endothelial growth factor (^{64}Cu-DOTA-VEGF) is a radiolabeled molecular imaging agent

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developed for positron emission tomography (PET) imaging of tumor angiogenesis and guiding antiangiogenic treatment (1). ^{64}Cu is a positron emitter with a half-life ($t_{½}$) of 12.7 h.

Angiogenesis is a process of development and growth of new blood vessels from pre-existing vessels (2). Tumor growth depends on the formation of new blood vessels from this process. Normal angiogenesis is orderly and highly regulated, whereas tumor angiogenesis is chaotic and irregular. Abnormal angiogenesis is important in the carcinogenesis, growth and progression of human solid and hematologic tumors (3). VEGF is both a vascular growth factor and a vascular permeability factor, and it plays a central and critical role in the regulation of angiogenesis (4). VEGF induces proliferation, sprouting, migration, and tube formation of endothelial cells (5). Different forms of VEGF bind to receptors that exhibit a tyrosine-kinase activity (2). VEGF and VEGF receptors (VEGFR) have been shown to be upregulated in many tumors.

VEGF is a dimeric glycoprotein of 36–46 kDa that has been implicated in several steps throughout the process of angiogenesis (3). The VEGF family currently consists of 6 growth factors and 3 high-affinity tyrosine-kinase receptors. The specific action of VEGF on the endothelial cells is mainly regulated by VEGFR1 and VEGFR2 (2). VEGF-A is the most well characterized member of the VEGF family and is composed of at least 7 isoforms due to alternative gene splicing. Located at chromosome 6p21.3, VEGF-A is encoded by 8 exons separated by 7 introns. These forms differ mainly in their bioavailability and can be characterized by heparin and heparin-sulfate binding domains encoded by exons 6 and 7. $VEGF_{121}$ (121 amino acids) and $VEGF_{165}$ (165 amino acids) isoforms have been shown to induce mitogenic and permeability-enhancing activity on endothelial cells. VEGF gene expression is upregulated by many stimuli and hormones. In cancer, VEGF is upregulated by the genetic events leading to malignant transformation. VEGF and its receptors are overexpressed in a variety of solid tumors (1).

Molecular imaging of angiogenesis offers serial non-invasive evaluation of various tumor parameters (6) PET imaging with the appropriate radiolabeled tracer targeted to angiogenic signaling pathways may allow the evaluation of specific aspects of tumor vascular biology. VEGF has been labeled with ^{111}In, ^{123}I and ^{99m}Tc as potential Single Photon Emission Tomography imaging agents (7). Cai et al. (1) prepared the first PET agent for imaging VEGF by labeling $VEGF_{121}$ with ^{64}Cu.

Synthesis

[PubMed]

Cai et al. (1) cloned and sequenced gene for $VEGF_{121}$ by polymerase chain reaction from human umbilical vein endothelial cells. The gene was inserted into the pET-32 vector downstream from the cleavable His_6 tag sequence. Bacterial cells were transformed and positive clones were selected for optimal protein expression. The protein was isolated and the His_6 tag was cleaved from $VEGF_{121}$. The final protein was purified and concentrated.

^{64}Cu labeling of $VEGF_{121}$ was performed through DOTA chelation (1, 8). DOTA was obtained commercially and activated by 1-ethyl-3-[3-(dimethylamino)-propyl] carbodiimide (EDC) and *N*-hydroxysulfonosuccimide (SNHS) at pH 5.5 for 30 min with a molar ratio of 10:5:4 (DOTA/EDC/SNHS). Without purification, the DOTA-hydroxysulfosuccinimidyl was cooled to 4°C and added to $VEGF_{121}$. The reaction mixture was adjusted to pH 8.5 with 0.1 N of sodium hydroxide and allowed to incubate overnight at 4° C. The DOTA-$VEGF_{121}$ conjugate was purified by a PD-10 column and concentrated. The final concentration was determined by ultraviolet absorbance at 280 nm. The number of DOTA molecules per $VEGF_{121}$ molecule was 4.3 ± 0.2 (n = 3). For radiolabeling, about 20 μg of this conjugate was added to 74 MBq (2.47 mCi) of ^{64}Cu chloride diluted in 300 μl of 0.1mol/L sodium acetate buffer. The reaction mixture was incubated for 1 h at 40°C with constant shaking. ^{64}Cu-DOTA-$VEGF_{121}$ was purified by chromatography. The ^{64}Cu labeling time, including the final purification was 90 ± 10 min (n = 5), and the radiolabeling yield was 87.4 ± 3.2%. The specific activity was 3.2 ± 0.1 GBq/mg (86.5 ± 2.7 mCi/mg). The radiochemical purity was >98%.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Cai et al. (1) used ^{125}I-$VEGF_{165}$ as the radioligand to perform cell binding assays of $VEGF_{121}$ and DOTA-$VEGF_{121}$ to PAE/KDR endothelial cells expressing VEGFR2. The 50% inhibitory concentrations (IC_{50}) were 1.02 nmol/L and 1.66 nmol/L for $VEGF_{121}$ and DOTA-$VEGF_{121}$, respectively. Stability studies of incubating ^{64}Cu-DOTA-$VEGF_{121}$ in complete mouse serum at 37°C showed no significant metabolite peak by high-performance liquid chromatography up to 24 h.

Animal Studies

Rodents

[PubMed]

MicroPET imaging studies of ^{64}Cu-DOTA-$VEGF_{121}$ were performed in mice bearing U87MG human glioblastomas. Each mouse received 5–10 MBq (0.14–0.27 mCi) of radioactivity in ~2–4 μg of $VEGF_{121}$. The radioactivity uptake was rapid and high in small tumors (tumor volume = 64.9 ± 24.6 mm^3, n = 3) with high VEGFR expression. The radioactivity levels measured as percentage of injected dose per g (% ID/g) were 14.9 ± 0.7, 16.3 ± 0.7, 16.3 ± 0.6, and 15.1 ± 0.8 at 2, 4, 16, and 23 h, respectively. The tumor was clearly visible as early as 1 h after injection. In comparison, the radioactivity uptake in the large tumors (tumor volume = 1,164.3 ± 179.6 mm^3, n = 3) with low VEGFR expression was very low at all time points. The radioactivity levels were ~3–4% ID/g and ~1–2% ID/g at the peripheral region and the necrotic center, respectively. The radioactivity levels were high in the kidney (33.0 ± 13.5% ID/g at 2 h) and liver (17.1 ± 3.2% ID/g at 2 h) in both groups of mice bearing small and large tumors. These

radioactivity levels dropped steadily over time. All other organs exhibited very low radioactivity levels. Biodistribution studies of mice at 23 h after administration confirmed that the PET quantification was a true reflection of organ radioactivity levels.

Blocking experiments were performed to test the specificity of ^{64}Cu-DOTA-VEGF$_{121}$ in the group of mice bearing small tumors (1). A dose of 100 μg of unlabeled VEGF$_{121}$ was injected into each mouse 30 min before the administration of ^{64}Cu-DOTA-VEGF$_{121}$. PET imaging showed the radioactivity uptake appeared to be noticeably lower than that of mice without blocking. The radioactivity levels in the tumor were significantly lower at all time points. Extrapolated from the figure in the article, the radioactivity levels (% ID/g) in small tumors with blocking were estimated to be ~9 (2 h), ~10 (4 h), ~11 (16 h), and ~10 (23 h), respectively.

Immunofluorescence staining and Western blot of tumor sections from studied mice confirmed their levels of VEGFR expression (1). The small blood vessels of the kidneys also appeared to contain a measurable level of VEGFR2. The authors suggested that the good correlation between *in vivo* PET and *ex vivo* results indicated that PET imaging with ^{64}Cu-DOTA-VEGF$_{121}$ could show the VEGFR expression level *in vivo*.

Other Non-Primate Mammals

[PubMed]

No publication is currently available.

Non-Human Primates

[PubMed]

No publication is currently available.

Human Studies

[PubMed]

On the basis of microPET quantification data of biodistribution and pharmacokinetics of ^{64}Cu-DOTA-VEGF$_{121}$ in rats, Cai et al. (1) estimated the radiation dosimetry in normal human organs. The radioactivity distribution in rats was similar to that in mice. ^{64}Cu-DOTA-VEGF$_{121}$ was primarily excreted by the liver. Only the blood, liver, and kidneys exhibited high levels of radioactivity. No appreciable activity was observed in the urinary bladder at any time point. This suggested that ^{64}Cu-DOTA-VEGF$_{121}$ had a very slow renal clearance, and that the radioactivity bound in the kidney was not excreted. The kidney was the critical organ with the highest estimated radiation-absorbed dose of 1.05 ± 0.27 mGy/MBq (3.87 ± 1.01 rad/mCi). The liver dose was estimated to be 0.12 ± 0.02 mGy/MBq (0.43 ± 0.07 rad/mCi). The whole-body absorbed dose was estimated to be 0.05 ± 0.01 mGy/MBq (0.19 ± 0.02 rad/mCi).

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