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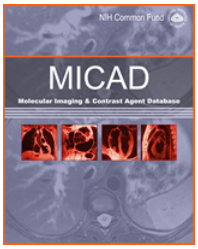

^{64}Cu-Labeled 1,4,7,10-Tetraazacyclododedane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid–conjugated vascular endothelial growth factor A isoform 121-gelonin fusion protein

^{64}Cu-DOTA-VEGF$_{121}$/rGel

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Chemical name:	^{64}Cu-Labeled 1,4,7,10-Tetraazacyclododedane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid–conjugated vascular endothelial growth factor A isoform 121-gelonin fusion protein	
Abbreviated name:	^{64}Cu-DOTA-VEGF$_{121}$/rGel	
Synonym:		
Agent Category:	Proteins	
Target:	Vascular endothelial growth factor receptor (VEGFR)	
Target Category:	Receptors	
Method of detection:	Positron emission tomography (PET)	
Source of signal / contrast:	Copper-64 (^{64}Cu)	
Activation:	No	
Studies:	• *In vitro* • Rodents	No structure is available.

Background

[PubMed]

^{64}Cu-Labeled 1,4,7,10-tetraazacyclododedane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid (DOTA)–conjugated vascular endothelial growth factor A isoform 121 (VEGF$_{121}$)-gelonin fusion protein (VEGF$_{121}$/rGel), abbreviated as ^{64}Cu-DOTA-VEGF$_{121}$/rGel, is an imaging agent developed by Hsu et al. for monitoring the targeting efficiency and treatment efficacy of the VEGF$_{121}$/rGel immunotoxin (1).

VEGFs are a group of five potent inducers of cell migration, invasion, vascular permeability, and neovascular formation (2). They act *via* three receptor tyrosine kinases: VEGFR-1, VEGFR-2, and VEGFR-3 (3). These receptors are overexpressed on the endothelial cells of tumor neovasculature and are almost undetectable in the

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endothelium of adjacent normal tissues. The critical role of the VEGF/VEGFR signal pathway in tumor angiogenesis has prompted great efforts in the development of antiangiogenic therapies, and agents have been tested by acting on different steps of the pathway, such as by binding to the VEGF ligand, inhibiting VEGFR tyrosine kinase, inhibiting downstream effectors (mammalian target of rapamycin inhibitors), and modulating VEGF production (4-6). These agents are highly effective against tumor growth in animal models when they are used alone; however, they seldom lead to tumor regression and exhibit insufficient efficacy in the clinical setting, although combination with chemotherapy has been shown to improve patient survival in certain tumor types. The most likely explanation for this phenominon is that tumor endothelial cells can adapt to antiangiogenic treatment and form functional vasculature that loses sensitivity to the inhibitors of VEGF/VEGFR (4, 6). It is hypothesized that VEGF/VEGFR-targeted therapy should be administrated before the development of a well-established vascular network.

Molecular imaging provides a means to reveal the mechanism underlying this phenomenon and to monitor the antiangionic therapy (1, 7). $VEGF_{121}$/rGel has been generated with $VEGF_{121}$, which is linked with recombinant plant toxin gelonin through a G_4S tether (4, 8-10). Gelonin is a member of the ribosome-inactivating protein family, which depurinates rRNA and other polynucleotide substrates and subsequently inhibits protein synthesis (11). A series of preclinical studies showed that $VEGF_{121}$/rGel could specifically inhibit the growth of tumor endothelial cells (8-10, 12). Like other immunotoxins, $VEGF_{121}$/rGel is also expected to be effective against tumors resistant to VEGF/VEGFR-targeting inhibitors if the tumor cells express sufficient levels of VEGFR (11). To monitor the VEGFR-targeting efficiency of $VEGF_{121}$/rGel with imaging techniques, Hsu et al. and Cho et al. labeled the $VEGF_{121}$/rGel with ^{64}Cu (^{64}Cu-DOTA-$VEGF_{121}$/rGel) and with $MnFe_2O_4$ nanoparticles ($VEGF_{121}$/rGel-MNPs), respectively (1, 7). Both imaging studies have concluded that noninvasive imaging with $VEGF_{121}$/rGel will be useful to monitor the treatment efficacy and to identify patients who may benefit from the $VEGF_{121}$/rGel therapy. This chapter summarizes data obtained with ^{64}Cu-DOTA-$VEGF_{121}$/rGel (1).

Related Resource Links:

VEGF/VEGFR-targeted imaging agents in MICAD

Articles on VEGF in Online Mendelian Inheritance in Man (OMIM)

VEGF-related compounds in PubChem Substance

VEGF-related nucleotide sequences

Synthesis

[PubMed]

Hsu et al. described the synthesis of ^{64}Cu-DOTA-$VEGF_{121}$/rGel (1). The synthesis, expression, and purification of the $VEGF_{121}$/rGel immunotoxin were performed as described previously by Veenendaal et al. (8). The molecular weight of $VEGF_{121}$/rGel was 84 kDa. $VEGF_{121}$/rGel was conjugated to DOTA to generate DOTA-$VEGF_{121}$/rGel. Labeling with ^{64}Cu was completed in the reaction of $^{64}CuCl_2$ and DOTA-$VEGF_{121}$/rGel for 1 h at 40°C.

The total time for ^{64}Cu-labeling of the DOTA-$VEGF_{121}$/rGel, including the final purification, was 90 ± 10 min (n = 3). The radiolabeling yield was 85.2 ± 9.2% on the basis of 37 MBq (1 mCi) ^{64}Cu per 25 μg DOTA-$VEGF_{121}$/rGel (n = 3). The specific activity of ^{64}Cu-DOTA-$VEGF_{121}$/rGel was 1.3 ± 0.1 GBq/mg (35.14 ± 2.7 mCi/mg), and the radiochemical purity was ≥98%. The number of DOTA molecules per $VEGF_{121}$/rGel molecule was 3.3 ± 0.1 (n = 4).

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

A cell-binding assay with VEGF$_{121}$/rGel and the DOTA-VEGF$_{121}$/rGel conjugate was performed with ^{125}I-VEGF$_{165}$ (specific activity, 74 TBq/mmol (2 kCi/mmol)) as the radioligand (1). Both VEGF$_{121}$/rGel and DOTA-VEGF$_{121}$/rGel without the metal inhibited the ^{125}I-VEGF$_{165}$ binding to PAE/KDR cells (porcine aortic endothelial cells transfected with cDNA of VEGFR2) in a dose-dependent manner. The 50% inhibition concentrations of VEGF$_{121}$/rGel and DOTA-VEGF$_{121}$/rGel were 24.5 nM and 40.6 nM, respectively, indicating that DOTA conjugation induced no significant change in the VEGF$_{121}$/rGel binding affinity. Western blot analysis (functional assay) of the VEGFR2 expression on PAE/KDR cells revealed a slight decrease in the expression level of phosphorylated VEGFR2 after DOTA conjugation. Increased expression levels of the phosphorylated VEGFR2 were observed at concentrations ≥5 nM for both VEGF$_{121}$/rGel and DOTA-VEGF$_{121}$/rGel.

Animal Studies

Rodents

[PubMed]

Positron emission tomography with ^{64}Cu-DOTA-VEGF$_{121}$/rGel was performed in athymic nude mice bearing intracranial tumors (n = 3) (1). Tumors were generated by intracranial injection into the right frontal lobe with 10^5 firefly luciferase-transfected U87MG human glioblastoma cells (U87MG-fLuc). The mice were intravenously injected with 5–10 MBq (0.14–0.27 mCi) ^{64}Cu-DOTA-VEGF$_{121}$/rGel and were imaged for up to 48 h after injection.

^{64}Cu-DOTA-VEGF$_{121}$/rGel exhibited high tumor accumulation and retention, as well as high tumor/background contrast from 1 h to 48 h after injection (1). Tumor accumulation at 1 h after injection was 5.8 ± 0.5% injected dose per gram (ID/g) (n = 3) and steadily increased, peaking at ~18 h after injection (11.8 ± 2.3% ID/g). At 46 h after injection, tumor uptake decreased to 8.4 ± 1.7% ID/g. There was no clear relationship between tumor size and tracer uptake. ^{64}Cu-DOTA-VEGF$_{121}$/rGel was cleared through both the hepatic and the renal pathways (data not shown). However, no evidence about the *in vivo* stability of this agent was reported.

A blocking study was carried out by injecting 200 μg VEGF$_{121}$ before injecting ^{64}Cu-DOTA-VEGF$_{121}$/rGel. Blocking with VEGF$_{121}$ resulted in a significant reduction in the ^{64}Cu-DOTA-VEGF$_{121}$/rGel uptake ($P < 0.05$), suggesting VEGFR-specific tumor uptake of the ^{64}Cu-DOTA-VEGF$_{121}$/rGel (1).

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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