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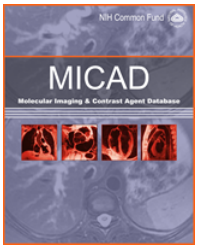

^{64}Cu-Labeled DOTA conjugated anti-epithelial membrane protein 2 minibody KS83

[^{64}Cu]-DOTA-KS83

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Chemical name:	^{64}Cu-Labeled DOTA-conjugated anti-epithelial membrane protein 2 minibody KS83	
Abbreviated name:	[^{64}Cu]-DOTA-KS83	
Synonym:		
Agent Category:	Antibody	
Target:	Epithelial membrane protein 2	
Target Category:	Antigen	
Method of detection:	Positron emission tomography (PET)	
Source of signal / contrast:	^{64}Cu	
Activation:	No	
Studies:	• *In vitro* • Rodents	Structure not available in PubChem.

Background

[PubMed]

Endometrial membrane protein-2 (EMP2) is a member of the growth-arrest specific protein 3/peripheral myelin protein 22 family of proteins, which are believed to play a role in the development of endometrial cancer in women (1). In addition, overexpression of EMP2 in endometrial cancer is considered to predict a negative outcome for the patient (2). Although the exact mechanisms of action of EMP2 are not known, it has been shown to modulate the translocation of molecules such as integrins, major histocompatibility complex-I, and glycophosphoinositol-linked proteins in the cell membrane (1). It has also been reported that EMP2 induces vascular endothelial growth factors in endometrial cancer tumors and promotes angiogenesis in lesions (3). In another study, it was shown that an engineered bivalent anti-EMP2 diabody suppressed the growth of HEC1A (human uterus/endometrium adenocarcinoma cell line) (4) and OVCARS5 (a human ovarian-endometrioid carcinoma cell line) (5) xenograft tumors in mice. Therefore, it was suggested that early detection and

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quantification of EMP2 could assist in the development of a suitable treatment regimen for patients suffering from endometrial cancer (6).

Fu et al. engineered an anti-EMP2 minibody (Mb, designated KS83; for a detailed structure of an Mb, see Fu et al. (6)), conjugated it to 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA), and labeled the conjugate with ^{64}Cu, a positron emitter ([^{64}Cu]-DOTA-KS83) (6). The non-radiolabeled Mb was investigated for its *in vitro* characteristics, and [^{64}Cu]-DOTA-KS83 was evaluated with positron emission tomography (PET) for the detection of tumors that overexpressed EMP2 in mice (6).

Related Resource Links

Minibody-related chapters in MICAD

EMP2 in Gene database (Gene ID: 2013)

Protein and mRNA sequences of human EMP2

Clinical trials on endometrial cancer

Synthesis

[PubMed]

The expression and purification of KS83 has been described elsewhere (6). The Mb was conjugated to DOTA and labeled with ^{64}Cu as detailed by Fu et al. (6). Approximately 3 molecules of DOTA were determined to be linked with each molecule of KS83 (6). The labeling reaction was reported to have an efficiency of 97%, but the radiochemical yield (RCY) and radiochemical purity (RCP) of the labeled Mb were not reported (6). The specific activity (SA) of [^{64}Cu]-DOTA-KS83 was 0.074 MBq/6.66 pmol (2 μCi/6.66 pmol).

For the biodistribution study, a ^{64}Cu-labeled DOTA-anti CD20 Mb ([^{64}Cu]-DOTA-CD20), an isotype Mb for the anti-EMP2 Mb, was used as a control (6). However, the RCY, RCP, and SA of the labeled Mb were not reported.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Western blot analysis of different tissues from mice showed that EMP2 was present mainly in the lungs and vas deferens of the animals (6). Immunohistochemistry of the different tissues confirmed that EMP2 was expressed only in the epididymis and lungs of the mice; it was not detected in any other tissues of the animals (6). An analysis of human tissue arrays with anti-EMP2 sera revealed that the protein was expressed in the alveolar epithelium of the lungs, pigmented epithelium of the eye, the endometrium, and the epithelia of the vagina and the fallopian tubes (6). Immunohistochemical analysis of human tissues showed that EMP2 was expressed primarily in the lungs and endometrial tumors, but it was not detected in the spleen (6).

Flow cytometric analysis of DOTA-KS83 with HEC1A cells transfected at the EMP2 gene (HEC1A/EMP2 cells; these cells overexpress EMP2) showed that cellular binding of the DOTA-conjugated Mb (EC_{50} = 0.30 nM) was comparable to that of the purified KS83 (EC_{50} = 0.36 nM) (6).

Animal Studies

Rodents

[PubMed]

The biodistribution of [^{64}Cu]-DOTA-KS83 was investigated in mice bearing HEC1A/EMP2 cell tumors and wild-type HEC1A cell tumors (express normal amounts of EMP2) or Ramos cell tumors (negative controls; do not express EMP2) (6). The animals (n = 5 mice/time point) were injected with 0.074 MBq (2 μCi) labeled Mb through the tail vein, and PET images of the rodents were acquired at 4 h postinjection (p.i.) and at 20 h p.i. Control animals (n = 2 mice) were injected with [^{64}Cu]-DOTA-CD20 and treated as described above. After the final imaging scan, the mice were euthanized and organs of interest were removed from the animals to determine the amount of radioactivity accumulated in the various tissues. Results obtained from this study were calculated as percent of injected dose per gram tissue (% ID/g) and are presented in Table 1. With [^{64}Cu]-DOTA-KS83, maximum radioactivity was detected in the kidneys (36.4 ± 8.3% ID/g), followed by the liver (18.1 ± 1.6% ID/g) and the lungs (5.5 ± 1.1% ID/g) at 20 h p.i. All other organs showed an accumulation of <5% ID/g at 20 h p.i. At 4 h p.i., the HEC1A/EMP2 tumors showed an uptake of 7.3 ± 0.5% ID/g, which increased to 9.7 ± 1.9% ID/g at 20 h p.i. The wild-type HEC1A cell tumors showed an accumulation of 5.8 ± 0.8% ID/g at 4 h p.i., which decreased to 4.2 ± 1.3% ID/g at 20 p.i. The Ramos cell tumors accumulated only 2.3 ± 0.5% ID/g radioactivity at 20 h p.i. (the amount of label present in these tumors at 4 h p.i. was not determined). With [^{64}Cu]-DOTA-KS83, 1.3-fold and 3-fold higher radioactivity accumulation was observed in the HEC1A/EMP2 tumors at 4 h p.i. and 20 h p.i., respectively, compared with [^{64}Cu]-DOTA-CD20. No blocking studies were reported.

The PET images showed that the HEC1A/EMP2 cell tumors and the wild-type HEC1A cell tumors were clearly visible at 4 h p.i. and 20 h p.i., but the Ramos tumors (EMP2-negative) showed almost no uptake of radioactivity even at 20 h p.i (6). At 4 h p.i., the uptake of label was observed in the lung, heart, liver, and kidneys of all the animals, and a high amount of the label was detected in the liver and kidney even at 20 h p.i. No blocking studies were reported.

From these studies, the investigators concluded that [^{64}Cu]-DOTA-KS83 is probably suitable for the detection and therapeutic monitoring of tumors that overexpress EMP2; however, more work is necessary before this ^{64}Cu-labeled Mb can be used in the clinic (6).

Table 1: Uptake of radioactivity from [^{64}Cu]-DOTA-KS83 and [^{64}Cu]-DOTA-CD20 in various tissues of mice bearing HEC1A/EMP2 cell tumors, wild-type HEC1A cell tumors, or Ramos cell tumors.

Tissues	Uptake of radioactivity (% ID/g)	
	4 h p.i.	**20 h p.i.**
HEC1A/EMP2 tumor	7.3 ± 0.5 (6.1 ± 0.1)	9.7 ± 1.9 (3.4 ± 0.6)
Wild type HEC1A tumor	5.8 ± 0.8 (NA)	4.2 ± 1.3 (NA)
Ramos tumor	ND (NA)	2.3 ± 0.5 (NA)
Liver	19.2 ± 5.4 (19.0 ± 3.1)	18.1 ± 1.6 (26.6 ± 0.9)
Kidney	47.1 ± 12.4 (33.1 ± 8.6)	36.4 ± 8.3 (24.2 ± 8.2)
Lung	12.5 ± 5.6 (12.1 ± 2.6)	5.5 ± 1.1 (6.2 ± 0.9)
Heart	9.2 ± 0.9 (8.3 ± 2.1)	4.3 ± 1.0 (6.2 ± 0.7)
Blood	18.1 ± 3.0 (13.2 ± 1.6)	3.7 ± 0.6 (3.1 ± 1.9)
Uterus	5.4 ± 0.8 (5.6 ± 2.7)	4.9 ± 1.6 (4.9 ± 0.6)

Numbers in parenthesis represent the uptake of radioactivity in the tissue from [^{64}Cu]-DOTA-CD20; NA: not available; ND: not determined. For complete data, see Fu et al. (6).

Other Non-Primate Mammals

[PubMed]

No reference is currently available.

Non-Human Primates

[PubMed]

No reference is currently available.

Human Studies

[PubMed]

No reference is currently available.

Supplemental Information

[Disclaimers]

No information is currently available.

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