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NLM Citation: Chopra A. ^{177}Lu-Labeled aglycosylated anti-L1-CAM monoclonal antibody chCE7. 2012 Jun 26 [Updated 2012 Jul 26]. In: Molecular Imaging and Contrast Agent Database (MICAD) [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2004-2013.
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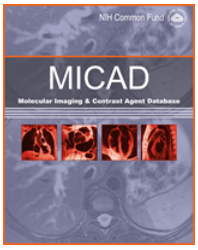

^{177}Lu-Labeled aglycosylated anti-L1-CAM monoclonal antibody chCE7

[^{177}Lu]-chCE7agl

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Created: June 26, 2012; Updated: July 26, 2012.

Chemical name:	^{177}Lu-Labeled aglycosylated anti-L1-CAM monoclonal antibody chCE7	
Abbreviated name:	[^{177}Lu]-chCE7agl	
Synonym:		
Agent Category:	Antibody	
Target:	L1-CAM antigen	
Target Category:	Antigen	
Method of detection:	Single-photon emission computed tomography (SPECT); gamma planar imaging	
Source of signal / contrast:	^{177}Lu	
Activation:	No	
Studies:	• *In vitro* • Rodents	Structure not available in PubChem.

Background

[PubMed]

Expression of the L1-cell adhesion molecule (L1-CAM) is reported to correlate with and is required for the progression and metastasis of many cancers, including that of the ovaries (1). Therefore, the L1-CAM has been targeted for the therapy of many cancers, as discussed in detail by Weidle et al. (2). The chimeric monoclonal antibody (mAb) chCE7, directed toward the L1-CAM, has been shown to have a very high affinity for the antigen (K_d ~1 pmol/L) and to inhibit the proliferation of ovarian cancer cells *in vitro* (3). However, in a preliminary clinical study it was shown that, although the ^{131}I-labeled mAb was suitable for the imaging of rapidly growing cancerous lesions in patients, it could not detect the large metastasized tumors in the individuals (4). In another study, ^{177}Lu- and $^{67/64}$Cu-labeled F(ab')$_2$ fragments of chCE7 were evaluated in nude mice for radioimmunotherapy (RIT) and positron emission tomographic imaging of intraperitoneal (i.p.) SKOV3 cell (SKOV3ip; of human ovarian cancer origin) tumors that express the L1-CAM antigen (5). Although the ^{177}Lu- and the $^{67/64}$Cu-labeled fragments of the mAb were able to detect the tumors at 24 h postinjection (p.i.), a very high proportion of radioactivity from both radiolabeled mAbs (34.5% of total injected dose per gram tissue (%

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ID/g) and 16.0% ID/g for ^{177}Lu and ^{67}Cu, respectively) was observed to accumulate in the kidneys of the animals (5). From these studies, the investigators concluded that a radiolabeled variant of the chCE7 that can clear rapidly from circulation and show low accumulation of radioactivity in the kidneys will have to be developed for the RIT of cancerous tumors that overexpress the L1-CAM antigen. To circumvent these problems, an aglycosylated form of chCE7 was engineered (chCE7agl) by substituting the asparagine residue at position 297 (a glycosylation site) with glutamine in the heavy chain of the mAb (3). A biodistribution study of ^{67}Cu-labeled chCE7agl in nude mice bearing SKOV3ip tumors showed that there was a high and persistent uptake of radioactivity in the lesions and that a single dose of [^{67}Cu]-chCE7agl reduced the growth of the tumor and prolonged the survival of the rodents (3). This study suggested that aglycosylation of chCE7 improved the pharmacokinetics, biodistribution, and therapeutic efficacy of the mAb.

To enhance the RIT efficacy of a mAb, it is important to label it with a radionuclide that is readily available, has a reasonable half-life, and emits medium-energy β-particles (6). The metallic radionuclide ^{177}Lu falls into this category (half-life, 6.7 days; mean range, 0.2 mm; %B = 100) and has been used widely to label mAbs and peptides for imaging and RIT studies in animals and humans [PubMed]. The chCE7agl mAb was labeled with ^{177}Lu ([^{177}Lu]-chCE7agl), and the *in vitro* and *in vivo* properties of the labeled mAb, including biodistribution, were investigated in normal nude mice or nude mice bearing SKOV3ip tumors (1, 6).

Related Resource Links

Chapters related to CAMs in MICAD

Clinical trials related to CAMs

Human L1-CAM protein and mRNA (variant 3) sequences

L1-CAM in Online Mendelian Inheritance in Man Database (OMIM)

Information on L1-CAM in Kyoto Encyclopedia of Genes and Genomics (KEGG)

Synthesis

[PubMed]

The chCE7agl mAb was produced in and purified from HEK-293 cells as described elsewhere (6). The mAb was conjugated to 1,4,7,10-tetraazacyclododecane-N-N'-N''-N'''-tetraacetic acid (DOTA) for labeling with ^{177}Lu, and the number of chelator molecules attached to each molecule of chCE7agl was determined with a spectrometric method as described by Knogler et al. (6). The chelator/mAb ratios of the three DOTA-chCE7agi conjugates were determined to be 7, 12, and 15, respectively, and were dependent on the molar excess ratio of DOTA (with respect to chCE7agl) used in the coupling reaction (6). After labeling with ^{177}Lu, the specific activities of chCE7agl conjugated to 7, 12, or 15 groups of DOTA were 30 MBq/6.66 nmol (0.8 mCi/6.66 nmol), 106 MBq/6.66 nmol (2.82 mCi/6.66 nmol), and 103 MBq/6.66 nmol (2.78 mCi/6.66 nmol), respectively. The radiochemical yields of the labeled mAbs ranged from 4.5% to 59.0%. The radiochemical purity (RCP) of the final product was not reported.

In another publication, an average of 4.2 DOTA molecules were reported to be conjugated with each molecule of chCE7agl (1). After labeling with ^{177}Lu, the immunoconjugate had a specific activity ranging from 52–153 MBq/6.66 nmol (1.4–4.1 Ci/6.66 nmol), and the labeling efficiency of the reaction was between 62% and 87%. The RCP and the stability of the labeled mAb were not reported.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

The stability of the immunoconjugate containing 12 chelator molecules was determined by incubating 1 pmol [^{177}Lu]-chCE7agl in 1 ml human plasma for 24 h at 37°C (6). The labeled mAb remained stable under these experimental conditions (no release of ^{177}Lu or denaturation of the complex) as determined with high-performance liquid chromatographic analysis and size-exclusion chromatography (6).

In a competition assay with ^{125}I-labeled chCE7agl as the ligand and SKVO3ip cells as the target, the IC_{50} values of chCE7agl conjugated to 7, 12, or 15 DOTA molecules were determined to be 0.79 pmol, 1.36 pmol, and 6.58 pmol, respectively (6).

Animal Studies

Rodents

[PubMed]

Knogler et al. investigated the biodistribution of [^{177}Lu]-chCE7agl coupled to different numbers of DOTA groups in the blood and liver of normal nude mice (Table 1) (6). The rodents (n = 3 mice/group) were injected with ~0.3 MBq (8.1 µCi) of the labeled conjugates through the tail vein, and the animals were euthanized at either 24 h p.i. (for immunoconjugates with 12 or 15 DOTA) or at 48 h p.i. (for the immunocomplex with 7 DOTA). Subsequently, the blood and liver of the mice were collected to determine the amount of radioactivity accumulated in these tissues.

Table 1: Accumulation of radioactivity with different DOTA-chCE7agl conjugates in the blood and liver of normal nude mice (6).

DOTA/chCE7agl ratio	Accumulated radioactivity (% ID/g)		Liver/Blood ratio	Time p.i. (h)
	Blood	Liver		
7	12.03 ± 2.22	3.99 ± 1.10	0.33	48
12	12.16 ± 4.13	14.67 ± 8.88	1.20	24
15	1.81 ± 0.36	30.96 ± 0.79	17.10	24

From Table 1 it is clear that the DOTA/mAb ratio influenced the liver/blood ratio of the immunoconjugates. Among the different DOTA-chCE7agl conjugates, only conjugates with 7 and 12 DOTA molecules showed slow clearance from the blood and had a low accumulation in the liver. The conjugate with 15 DOTA coupled to the mAb exhibited rapid clearance from blood and had a very high accumulation in the liver (liver/blood ratio = 17.10).

Fischer et al. investigated the biodistribution of [^{177}Lu]-chCE7agl in CD1-*Foxn*^{nu} mice bearing intraperitoneal SKOV3ip1 tumors (1). The animals (n = at least 4 mice/time point) were injected with 4 MBq (~110 µCi; ~200 pmol) labeled mAb through the tail vein and euthanized at time points ranging from 24 h p.i. (1 d p.i.) to 336 h p.i. (14 d p.i.). All the organs of interest, including blood, were harvested from the rodents to quantify the accumulation of radioactivity in the various tissues. At 24 h p.i., the amount of tracer in the blood, liver, and tumors was 12.5 ± 2.5% ID/g, 6.5 ± 1.8% ID/g, and 29.1 ± 6.3% ID/g, respectively. The peak accumulation of label in the tumors was 48.0 ± 8.1% ID/g at 168 h p.i. (7 d p.i.), and the amount of radioactivity in the blood and liver at this time point was 2.2 ± 0.8% ID/g and 3.1 ± 0.2% ID/g, respectively. At 168 h p.i., all other organs had an accumulation of <3.5 ± 1.2% ID/g (spleen) of the tracer. A high level of the label was present in the tumors even at 336 h p.i. (22.8 ± 5.2% ID/g), whereas all other organs, except the spleen, showed an accumulation of <2.1 ± 0.07% ID/g in the tissues. The uptake of radioactivity in the spleen was 6.4 ± 1.0% ID/g at this time point. No blocking studies were reported.

For single-photon emission computed tomographic (SPECT) and computed tomographic (CT) imaging, CD1-*Foxn*^{nu} mice (the number of animals was not reported) bearing either subcutaneous (s.c.; located on the shoulders) or SKOV3ip1 cell tumors were injected with [^{177}Lu]-chCE7agl through a lateral tail vein (1). Mice without tumors served as controls and were injected with a similar amount of radioactivity through the same route as the test animals. SPECT/CT images acquired from the animals at 48 h p.i. showed that the s.c and the i.p. tumors accumulated a high amount of the tracer and were clearly visible in the animals. In addition, disseminated tumor nodules were also visible in animals with the i.p. tumors. No radioactivity was evident in the blood pool or other organs of the control mice or animals bearing the s.c. or i.p. tumors. No blocking studies were reported to determine saturable binding.

From these studies, the investigators concluded that [^{177}Lu]-chCE7agl can be used for the RIT of tumors that overexpress the L1-CAM antigen in rodents (1).

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.

Supplemental Information

[Disclaimers]

No information is currently available.

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