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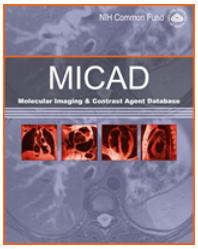

[^{111}In]-Labeled monovalent Fab fragment of chimeric monoclonal antibody cG250 directed against carbonic anhydrase IX

[^{111}In]-DOTA-Fab-cG250

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Chemical name:	[^{111}In]-Labeled monovalent Fab fragment of chimeric monoclonal antibody cG250 directed against carbonic anhydrase IX	
Abbreviated name:	[^{111}In]-DOTA-Fab-cG250	
Synonym:	[^{111}In]-DO3A-Fab-cG250; [^{111}In]-Fab-cG250	
Agent Category:	Antibody	
Target:	Carbonic anhydrase IX	
Target Category:	Enzyme	
Method of detection:	Single-photon emission tomography (SPECT); gamma planar imaging	
Source of signal / contrast:	^{111}In	
Activation:	No	
Studies:	• *In vitro* • Rodents	Structure not available in PubChem.

Background

[PubMed]

A common feature of most solid cancerous tumor types is the presence of hypoxic conditions (1) and the overexpression of carbonic anhydrase IX (CA IX), a transmembrane cell-surface enzyme that is known to regulate the pH and adhesion of tumor cells (2). Hypoxic tumors are often resistant to radio- and chemotherapy, have a high metastatic potential, and usually predict a poor outcome for the cancer patient (3). Although several methods (invasive and noninvasive) are available for the detection of hypoxia in tumors, including the use of radiolabeled small molecules, these methods are not completely reliable because they either yield variable diagnoses or have functional limitations due to incomplete penetration of tumors and fail to detect hypoxia in all tumor types (3, 4). Because CA IX is overexpressed in most solid tumors, it is considered to be a hypoxia biomarker, and targeting the CA IX for the detection of hypoxic tumors is of great interest to investigators (1, 3-5). A ^{131}I-labeled murine monoclonal antibody (mAb) that targets the CA IX, designated G250, was

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developed and evaluated for the radiotherapy of metastatic renal cell carcinoma (RCC) patients, but no major responses were observed because the individuals developed immunity to the mAb (6). Subsequently, a ^{131}I-labeled chimeric form of G250, [^{131}I]-cG250, was developed and evaluated as an immunotherapeutic agent for the treatment of RCC (7). cG250 has been labeled with other nuclides (such as ^{89}Zr, ^{177}Lu, ^{90}Y, etc.) and has been used in preclinical studies in rats (8) and for the treatment of RCC (7). However, only minor responses were observed in the clinical investigations, and dose escalation studies are ongoing (7).

Radiolabeled antibodies (Abs) have a limited ability to detect or treat cancer because these agents show only a peripheral penetration of solid tumors (due to a large size, ~150 kDa) and leave many neoplastic cells in the lesion untreated (9). In addition, Abs have prolonged blood circulation and present a high radiation dose risk to the bone marrow (10). In comparison, the smaller monovalent Fab (~50 kDa) and the divalent F(ab')$_2$ (~100 kDa) fragments derived from the parent Ab exhibit better tumor penetration and a shorter circulating half-life and are likely to yield better results if used to detect or treat solid malignant tumors (9). Between the two fragment types, the divalent F(ab')$_2$ fragments may be more useful for the detection or treatment of malignant tumors because they have a higher affinity for the antigen (11). With these observations in mind, a divalent F(ab')$_2$ fragment of cG250 was developed, labeled with ^{131}I, and compared with the intact [^{131}I]-cG250 Ab for its pharmacokinetic behavior and its ability to target tumors in mice and RCC patients (10). However, from this study the investigators concluded that the intact Ab was superior to the divalent fragment for targeting the RCC tumors. A clinical trial to investigate the safety of a ^{124}I-labeled version of cG250 in patients with renal masses has been reported (12). In addition, cG250 is also under evaluation in several other clinical trials. Recently, ^{89}Zr-labeled F(ab')$_2$ fragments of cG250 were shown to be suitable for the visualization of hypoxic head and neck cancer xenograft tumors in mice (5).

Brouwers et al. compared the use of [^{111}In]-isothiocynate-diethylenetriamine pentaacetic acid-cG250 and [^{131}I]-cG250 for the detection of RCC metastases in five patients and concluded that the former tracer was superior to the latter for visualization of the tumors (13). In another study involving three patients, it was shown that neither ^{131}I-labeled cG250 nor ^{111}In-labeled cG250 were suitable for the radioimmunotherapy of biliary cancer (14). In a recent study using 1,4,7,10-tetraazacyclododecane-*N*,*N*',*N*'',*N*'''-tetraacetic acid (DOTA) as a nuclide conjugating agent, ^{111}In-labeled cG250 Ab ([^{111}In]-DOTA-cG250) and its Fab ([^{111}In]-DOTA-Fab-cG250) and F(ab')$_2$ ([^{111}In]-DOTA-F(ab')$_2$-cG250) fragments were generated and compared for their biodistribution and detection of hypoxic HT-29 cell (of human colorectal adenocarcinoma origin) xenograft tumors in mice (1).

This chapter details the studies performed with [^{111}In]-DOTA-Fab-cG250. Studies performed with [^{111}In]-DOTA-cG250 (15) and [^{111}In]-DOTA-F(ab')$_2$-cG250 (16) are discussed in separate chapters of MICAD (www.micad.nih.gov).

Other Sources of Information

Clinical trials on carbonic anhydrase IX inhibitors

Human carbonic anhydrase IX in Entrez Gene (Gene ID 768)

Protein and mRNA sequence of human carbonic anhydrase IX

Crystal structure of the human carbonic anhydrase IX catalytic domain

Human carbonic anhydrase IX in Online Mendelian Inheritance in Man (OMIM) database

Hypoxia response in National Cancer Institute-Nature Pathways Interaction Database

Synthesis

[PubMed]

The production and labeling of the cG250 Fab fragment with ^{111}In was described in detail by Carlin et al. (1). On average, 1.9 ± 0.1 molecules of DOTA were reported to be conjugated to each molecule of Fab-cG250 (equal to 1.3% DOTA w/w). The ^{111}In-labeling efficiency of the cG250 Fab fragment was >90% with a radiochemical purity of >99.9% and a specific activity of 370 MBq/mg (~1.5 Ci/mg).

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Using SKRC-38 cells (of human RCC origin) under *in vitro* conditions, the immunoreactivity of [^{111}In]-DOTA-Fab-cG250 was >90% with a B_{max} of 118,000 ± 21,000 binding sites/cell (1). Under the same experimental conditions, the B_{max} values for the cG250 and its F(ab')$_2$ fragment were 120,000 ± 22,000 and 114,000 ± 19,000 binding sites/cell, respectively (1). [^{111}In]-DOTA-Fab-cG250 was reported to have a K_d of 14.05 ± 0.47 nM for the CA IX on the SKRC-38 cells. In comparison, the K_d values for the ^{111}In-labeled cG250 and the F(ab')$_2$ fragment were 2.48 ± 0.04 and 1.76 ± 0.08 nM, respectively.

The tumor uptake of radioactivity from the labeled Fab, cG250, and the F(ab')$_2$ fragment was confirmed with *ex vivo* autoradiography of the tumor sections (1). In addition, the expression of CA IX and the occurrence of hypoxic conditions in the tumor sections were confirmed with immunohistochemical and pimonidazole staining procedures, respectively.

Animal Studies

Rodents

[PubMed]

The biodistribution of [^{111}In]-DOTA-Fab-cG250 was studied in *nu/nu* nude mice (n = 4–5 animals/group per time point) bearing hypoxic HT-29 colorectal tumor xenografts as described by Carlin et al. (1). The animals were injected with the ^{111}In-labeled Fab fragment through the tail vein and euthanized at preselected time points (ranging from 6 h to 7 days post-injection (p.i.)) to determine the amount of radioactivity accumulated in the tumors and the major organs. Data generated from the study were presented as percent injected dose per gram tissue (% ID/g).

The tumor uptake of radioactivity from the labeled Fab fragment was 3.6 ± 1.3% ID/g and 3.5 ± 1.7% ID/g at 6 h and 24 h p.i., respectively. The tumor/muscle (TM) and tumor/blood (TB) ratios with the labeled monovalent fragment were 4.8 and 2.8, respectively, at 6 h p.i., and these ratios increased to 6.7 and 16.6, respectively at 24 h p.i., which were lower than the ratios observed with [^{111}In]-DOTA-cG250 at 7 days p.i. (see below). Compared with the labeled Fab fragment, the accumulation of radioactivity in the tumors with the ^{111}In-labeled F(ab')$_2$ fragment was higher at the two time points, with 7.6 ± 1.4% ID/g and 9.3 ± 2.1% ID/g at 6 h and 24 h p.i., respectively. The TM and TB ratios with the divalent tracer were 8.9 and 4.6, respectively at 24 h p.i. The tumor uptake of ^{111}In-labeled cG250 was 20.1 ± 4.8% ID/g at 2 days p.i. and increased to 26.4 ± 5.7% ID/g at day 7. In general, the tumor/non-tumor (TNT) ratios with [^{111}In]-DOTA-cG250 increased for all tissues up to 7 days p.i., except for the liver and spleen, and little change in the TNT ratio for these organs was apparent during this period. At 7 days p.i., the TM and TB ratios with the labeled cG250 were 69 and 6.6, respectively, indicating a slow washout of radioactivity from these organs. The two Ab fragments showed ~10-fold lower tumor uptake and a similar increase in kidney accumulation of radioactivity compared with [^{111}In]-DOTA-cG250. A high

accumulation of radioactivity in the kidney was expected because the Fab and F(ab')$_2$ fragments (or their breakdown products) are known to be excreted through the urinary route and these organs are known to have a high expression of CA IX. These observations suggested that clearance of the [^{111}In]-DOTA-Fab-cG250 and the [^{111}In]-DOTA-F(ab')$_2$-cG250 fragments from blood was faster than that of the intact [^{111}In]-DOTA-cG250 Ab. These results indicated that cG250 had a long circulation time compared with the Fab and F(ab')$_2$ fragments and that the tumor had a superior retention of the labeled Ab compared to either of its fragments. No blocking studies were reported.

From these studies, the investigators concluded that imaging with [^{111}In]-DOTA-cG250 at 7 days p.i. was a better and more sensitive method for the detection of CA IX in hypoxic tumors in a murine model compared with its ^{111}In-labeled Fab or F(ab')$_2$ fragments (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.

Supplemental Information

[Disclaimers]

No information is currently available.

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