^{68}Ga-Labeled alanine derivatives of 1,4,7,10-tetraazacyclododecane-1,7-diacetic acid and 1,4,7,10-tetraazacyclododecane-1,4,7,-triacetic acid

^{68}Ga-21, ^{68}Ga-22

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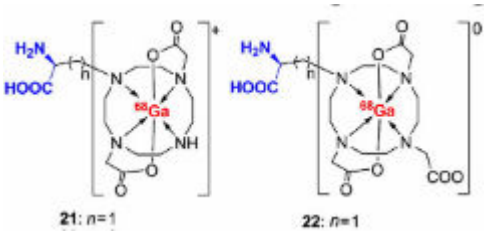
Chemical name:	^{68}Ga-Labeled alanine derivatives of 1,4,7,10-tetraazacyclododecane-1,7-diacetic acid and 1,4,7,10-tetraazacyclododecane-1,4,7,-triacetic acid
Abbreviated name:	^{68}Ga-21, ^{68}Ga-22
Synonym:	^{68}Ga-DO2A-alanine, ^{68}Ga-DO3A-alanine
Agent Category:	Others (amino acid)
Target:	Amino acid transporters
Target Category:	Transporters
Method of detection:	Positron emission tomography (PET)
Source of signal / contrast:	Gallium-68 (^{68}Ga)
Activation:	No
Studies:	• *In vitro* • Rodents



Structures of ^{68}Ga-21 and ^{68}Ga-22 by Shetty et al. (1).

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Background

[PubMed]

The ^{68}Ga-labeled alanine derivatives of 1,4,7,10-tetraazacyclododecane-1,7-diacetic acid (DO2A) and 1,4,7,10-tetraazacyclododecane-1,4,7,-triacetic acid (DO3A), abbreviated as ^{68}Ga-21 and ^{68}Ga-22, respectively, were synthesized by Shetty et al. for positron emission tomography (PET) of cancer cells (1).

Radiolabeled amino acids represent a diverse class of tracers that target the increased amino acid transport in cancer cells (2, 3). To date, >20 distinct amino acid transporters have been identified in mammalian cells, and these transporters differ in terms of substrate specificity, tissue expression patterns, sodium and other ion dependence, pH sensitivity, and transport mechanism (4, 5). Because of increased demand for amino acids in malignant cells, some transporters have been shown to be overexpressed in different types of tumors, and the process of amino acid transport is relatively fast (2, 6, 7). These features make tumor imaging with amino acid tracers possible within 20 min. Indeed, there is growing evidence that radiolabeled amino acids have the potential to overcome some of the limitations of 2-deoxy-2-[^{18}F]fluoro-D-glucose ([^{18}F]FDG) in tumor imaging, especially in the imaging of primary and recurrent brain tumors, neuroendocrine tumors, and prostate cancers (2, 3, 8). Different studies also showed that radiotracers that target different amino acid transporters exhibit different imaging properties that may provide unique biological information of tumors (1, 2, 7).

The first group of widely investigated amino acids is the analogs of phenylalanine and tyrosine (2, 3). Because of their bulky neutral side chains, these natural amino acids are the substrates of system L transporters and have been proven to be useful for tumor imaging, particularly for brain tumors. The limitation common to most of the natural amino acids is the susceptibility to *in vivo* metabolism, which decreases tumor specificity and complicates kinetic analysis. Because none of the natural amino acids contain fluorine or iodine, labeling with fluorine-18 or iodine-123 can change the biochemical properties compared to the parent amino acids. These shortcomings associated with natural amino acids can be partially overcome by using non-natural amino acids. Typically, non-natural amino acids are neither metabolized nor readily incorporated into protein *in vivo* (2, 3, 7). One group of non-natural amino acids is á,á-dialkyl amino acids, which are generated by substituting the á-carbon hydrogen of natural amino acids with a methyl group or other alkyl chains. These amino acids are primarily the substrates of system A transporters. The second group is alicyclic amino acids, which are á,á-dialkyl

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amino acids with side chains bonded covalently to each other to form a cyclic ring. These amino acids are the substrates of system L transporters. The third group is non-natural proline derivatives, which exhibit different transport selectivity. One challenge in developing amino acid radiotracers is to overcome the low selectivity and the decreased recognition after radiolabeling to specific transporters (1-3). Another challenge is the low uptake of amino acid agents in tumors, which leads to less sensitivity for tumor detection than with [^{18}F]FDG (8).

Shetty et al. synthesized a group of ^{68}Ga-labeled alanine and lysine derivatives of 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA), 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA), DO2A, and DO3A (1, 7). The four bifunctional chelating agents have similar sizes, but they differ in the net charges because of the different numbers of pendent carboxylate arms. The amino acids have been conjugated to one of the carboxylate arms, and the nitrogen atoms in the heterocyclic ring are presumed to coordinate with metals to form chelates. Biodistribution studies and PET imaging indicate the structure–activity relationship of the amino acid derivatives, and the selective uptakes of these compounds by different cancer tissues might provide an insight on the different modes of amino acid uptake by cancer cells (1, 7).

This chapter summarizes the data obtained with ^{68}Ga-labeled alanine derivatives: ^{68}Ga-21 (^{68}Ga-DO2A-alanine) and ^{68}Ga-22 (^{68}Ga-DO3A-alanine). These alanine derivatives were comparatively analyzed with the corresponding homoalanine derivatives (^{68}Ga-23 and ^{68}Ga-24, respectively) (1).

Related Resource Links:

Amino acid transporter-targeted imaging agents in MICAD

Amino acid transporter articles in Online Mendelian Inheritance in Man (OMIM)

Amino acid transporter related clinical trials in ClinicalTrials.gov

Amino acid transporter related bioassays in PubChem BioAssay

Synthesis

[PubMed]

Shetty et al. synthesized the alanine derivatives, DO2A-alanine (compound 21) and DO3A-alanine (compound 22), by reacting the protected L-serine â-lactone with the compounds 1,4,7,10-tetracyclododecene-1,7-bis(*tert*-butyl acetate) or 1,4,7,10-tetracyclododecene-1,4,7-tris(*tert*-butyl acetate), respectively (1).

Radiolabeling of the derivatives with ^{68}Ga was carried out at pH 3–4. The labeled reaction mixture was passed through a cartridge to remove the free $^{68}Ga^{3+}$. The labeled agents were analyzed with radiographic thin-layer chromatography. The labeling efficiencies were >97% for both ^{68}Ga-21 (^{68}Ga-DO2A-alanine) and ^{68}Ga-22 (^{68}Ga-DO3A-alanine). No free ^{68}Ga was found after purification. DOTA without an amino acid residue was

labeled with ^{68}Ga under the same conditions (^{68}Ga-DOTA) and was used as the control for *in vitro* cell uptake study. The specific activities and stabilities of ^{68}Ga-21, ^{68}Ga-22, and ^{68}Ga-DOTA were not reported (1).

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Cell uptake assays were performed after incubation of Hep3B (human hepatoma) and U87MG (human glioma) cells with 10 ìCi (0.37 MBq) of each agent at 37°C for various times (1). Both ^{68}Ga-21 and ^{68}Ga-22 exhibited greater uptakes by cells than the control ^{68}Ga-DOTA. ^{68}Ga-22 showed a higher uptake than ^{68}Ga-21 in both cell lines.

Comparative analysis was performed for the cell uptake between alanine and homoalanine derivatives (1). The results showed that all agents (^{68}Ga-21 to ^{68}Ga-24) exhibited higher uptake than ^{68}Ga-DOTA in both cell lines. ^{68}Ga-22 had the highest uptake in U87MG cells, whereas ^{68}Ga-23 had the highest uptake in Hep3B cells. These results may represent specific uptakes of the amino acid derivatives by cancer cells due to different expressions of various amino acid transporters on cell surfaces. Shetty et al. did not perform blocking experiments, considering that many amino acid derivatives can be transported simultaneously by several different types of transporters and the cell uptake cannot be completely blocked by a single inhibitor (1).

Animal Studies

Rodents

[PubMed]

The biodistribution patterns of ^{68}Ga-21 and ^{68}Ga-22 were investigated at 10, 30, 60, and 120 min after tail vein injection of each agent (10 ìCi (0.37 MBq)) in mice bearing CT-26 colon cancer xenografts (n = 3–4 mice/time point for each agent) (1). The initial tumor uptakes at 10 min after injection was 2.88% and 3.22% injected dose per gram of tissue (ID/g) for ^{68}Ga-21 and ^{68}Ga-22, respectively, which then decreased until 60 min (1.05% for ^{68}Ga-21 and 2.44% for ^{68}Ga-22) and remained almost constant until 120 min after injection (1.08% for ^{68}Ga-21 and 2.98% for ^{68}Ga-22). The tumor/muscle and tumor/blood ratios peaked at 120 min after injection. The tumor/muscle ratios for ^{68}Ga-21 and ^{68}Ga-22 were 2.23 and 2.46 at 120 min, respectively. The tumor/blood ratios for ^{68}Ga-21 and ^{68}Ga-22 were 0.56 and 0.49 at 120 min, respectively.

PET imaging was performed at 30 min after tail vein injection (0.37 MBq (0.01 mCi)) and showed that ^{68}Ga-21 had higher tumor uptake and standardized uptake value (SUV) than ^{68}Ga-22.

Comparative analysis was also performed for the biodistribution and PET imaging data between alanine and homoalanine derivatives (1). Comparison for the biodistribution showed that all of the alanine and homoalanine derivatives (^{68}Ga-21 to ^{68}Ga-24)

presented high kidney uptake and low liver and intestine uptake, indicating that the major excretion route of these agents is the kidney. The tumor uptake of alanine derivatives (^{68}Ga-21 and ^{68}Ga-22) was higher than that of homoalanine derivatives (^{68}Ga-23 and ^{68}Ga-24) at both 30 min and 60 min. However, homoalanine derivatives had higher tumor/nontumor ratios than alanine derivatives at all investigated time points. Similarly, high kidney and bladder activities were observed for all agents in PET imaging. The alanine derivatives showed higher tumor uptakes than the homoalanine derivatives. The SUV for all agents was lower than that of [^{18}F]FDG, which might be the result of slow uptake by cancer tissues and rapid excretion through the kidneys. However, tumor/nontumor SUV ratios were high enough to obtain tumor images. ^{68}Ga-24 showed the highest tumor/nontumor SUV ratio (3.9 ± 0.3), followed by ^{68}Ga-21 (3.1 ± 0.2), ^{68}Ga-22 (2.8 ± 0.2), and ^{68}Ga-23 (2.3 ± 0.2) at 30 min after injection. No studies of the mechanism were presented.

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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