^{68}Ga-Labeled β-aminoalanine, γ-aminohomoalanine, and ε-aminolysine conjugates of 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid

^{68}Ga-2a-c

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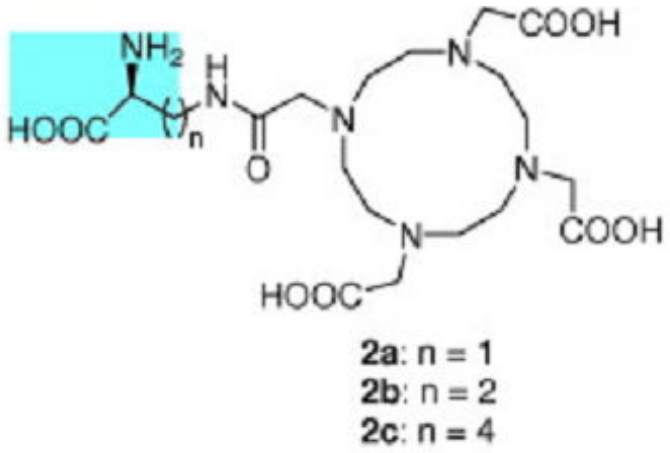
Chemical name:	^{68}Ga-labeled β-aminoalanine, γ-aminohomoalanine, and ε-aminolysine conjugates of 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid
Abbreviated name:	^{68}Ga-2a-c
Synonym:	^{68}Ga-2a, ^{68}Ga-2b, ^{68}Ga-2c
Agent Category:	Others (amino acid)
Target:	Amino acid transporters
Target Category:	Transporters
Method of detection:	Positron emission tomography (PET)
Source of signal / contrast:	Gallium-68 (^{68}Ga)
Activation:	No
Studies:	• *In vitro* • Rodents



Structure of 2a-c (n = 1, β-aminoalanine; n = 2, γ-aminohomoalanine; and n = 4, ε-aminolysine) by Shetty et al. (1).

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Background

[PubMed]

The ^{68}Ga-labeled â-aminoalanine, ã-aminohomoalanine, and å-aminolysine conjugates of 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA), abbreviated as ^{68}Ga-2a, ^{68}Ga-2b, and ^{68}Ga-2c, respectively, were synthesized by Shetty et al. for positron emission tomography (PET) of tumors (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 (1, 2, 6). 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, 7). 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, 8).

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 and retaining key biochemical properties are also challenging. 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* (1-3). 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 amino acids with side chains

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bonded covalently to each other to form a cyclic ring. These amino acids are the substrates of system L transporters. The third group is the 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 (2, 3, 8). 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 (7).

Shetty et al. synthesized a group of ^{68}Ga-labled alanine and lysine derivatives of DOTA, 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA), 1,4,7,10-tetraazacyclododecane-1,7-diacetic acid (DO2A), and 1,4,7,10-tetraazacyclododecane-1,4,7,-triacetic acid (DO3A) (1, 8). The four bifunctional chelating agents have similar sizes, but they differ in the net charges and stability 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, 8).

This chapter summarizes the data obtained with ^{68}Ga-labeled DOTA derivatives: ^{68}Ga-2a (^{68}Ga-DOTA-â-aminoalanine), ^{68}Ga-2b (^{68}Ga-DOTA-ã-aminohomoalanine), and ^{68}Ga-2c (^{68}Ga-DOTA-å-aminolysine). These DOTA derivatives were comparatively analyzed with the corresponding NOTA derivatives (^{68}Ga-1a, ^{68}Ga-1b, and ^{68}Ga-1c, 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]

The protected â-amino-L-alanine was synthesized from *N-tert*-butyl-L-serine methyl ester. Conjugation of DOTA with protected â-amino-L-alanine was performed in aqueous solution using 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride as a coupling reagent to obtain the alanine conjugate. Hydrolysis of the protected esters using LiOH, followed by hydrochloric acid, resulted in the formation of compound 2a (DOTA-â-aminoalanine) as a hydrochloride salt.

The ã-amino-L-homoalanine was purchased, and the å-aminolysine was synthesized from $N^{á}$-boc-$N^{å}$-benzyloxycarbonyl-lysine by *tert*-butyl esterification and subsequent

deprotection. Conjugation of ã-amino-L-homoalanine and å-aminolysine with DOTA was performed separately with *N*,*N*'-dicyclohexylcarbodiimide as a coupling agent and pyridine as a base. Further hydrolysis and deprotection resulted in the formation of compound 2b (DOTA-ã-aminohomoalanine) and compound 2c (DOTA-å-aminolysine), respectively. All final products were purified (1).

The DOTA and NOTA derivatives were labeled with ^{68}Ga either in a boiling water bath or at room temperature (1). The chelating agents, DOTA and NOTA, were also labeled with ^{68}Ga as controls for the *in vitro* protein binding and cell uptake studies. The labeling efficiency was >95% for all agents, and no free ^{68}Ga was found after purification. The specific activities of the purified agents were in the range 1.94–9.21 GBq/ìmol (52.43–248.92 mCi/µmol). Stability studies at room temperature confirmed the stability of all agents for >4 h.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Shetty et al. performed protein binding studies by incubating the agents with human serum at 37°C for 10 min and 1 h, respectively (1). ^{68}Ga-2c showed the lowest level of protein binding (2.44% at 10 min and 5.57% at 1 h), followed by ^{68}Ga-2b (6.40% at 10 min and 6.60% at 1 h) and ^{68}Ga-2a (9.87% at 10 min and 9.81% at 1 h). In comparison with the corresponding NOTA derivatives, all DOTA derivatives had higher levels of serum protein binding. Only the DOTA derivatives showed significant increases in protein binding over time. Of all NOTA and DOTA derivatives, ^{68}Ga-1b showed the lowest level of protein binding (1.71% at 10 min and 1.44% at 1 h) and ^{68}Ga-2a showed the highest level of binding (1). NOTA is known to form more stable chelate with ^{68}Ga than DOTA, and thus, ^{68}Ga might be transchelated to serum proteins such as transferrin from the less stable chelates of DOTA and its derivatives.

Cell uptake studies after incubation of the cells with each agent showed that all three agents had significantly higher uptakes than the control compounds (^{68}Ga-NOTA and ^{68}Ga-DOTA) in both Hep3B (a human hepatoma cell line) and CT-26 (a mouse colon cancer cell line) cells (1). Of the three agents, ^{68}Ga-2a had the highest uptake in Hep3B cells, while ^{68}Ga-2b had the highest uptake in CT-26 cells.

Animal Studies

Rodents

[PubMed]

The biodistribution of ^{68}Ga-2a, ^{68}Ga-2b, and ^{68}Ga-2c (10 ìCi/0.1 ml (0.37 MBq/0.1 ml)) was investigated after tail vein injection in mice bearing human colon cancer CT-26 xenografts (n = 4 mice/time point for each agent) (1). Mice were euthanized at different times (10, 30, 60, and 120 min) after injection, and counts were obtained with a ã-

scintillation counter. Tumor uptakes for all three agents were higher than those for most organs, except for the kidneys. Tumor/blood ratios were similar for all agents up to 60 min after injection but differed at 120 min. The tumor/blood ratios for ^{68}Ga-2a, ^{68}Ga-2b, and ^{68}Ga-2c were 0.72, 0.9, and 0.96, respectively, at 60 min after injection, and were 1.02, 1.41, and 1.73, respectively, at 120 min after injection. Tumor/muscle ratios were different among the agents. The highest tumor/muscle ratio was achieved by ^{68}Ga-2a (2.48), followed by ^{68}Ga-2b (1.65) and ^{68}Ga-2c (1.51) at 30 min after injection.

PET imaging in mice bearing human colon cancer CT-26 xenografts was performed at 30 min after injection of the agents (0.6–0.8 mCi (22.2–29.6 MBq)) through the tail vein (1). High levels of kidney and bladder activity were observed for all agents, indicating rapid renal excretion. Tumor uptakes were visualized by PET with all three agents (^{68}Ga-2a, ^{68}Ga-2b, and ^{68}Ga-2c), but ^{68}Ga-2a demonstrated the highest tumor/muscle ratio (5.9).

In comparison between DOTA and NOTA derivatives, all agents showed fast renal excretion and blood clearance, low nonspecific uptake in normal organs except for kidneys, and relatively high uptake in tumor tissues (1). In biodistribution studies, the tumor/blood ratios for NOTA-amino acid derivatives were higher than those for DOTA-amino acid derivatives at 2 h, although the ratios were similar for all six agents at 30 min after injection, which might be related to the higher serum protein binding by DOTA derivatives. In PET imaging, ^{68}Ga-1b demonstrated the highest tumor/muscle ratio (12.3), followed by ^{68}Ga-2a (5.9) and ^{68}Ga-2b (4.3). These results suggest that ^{68}Ga-1b is more favorable than others for use in PET imaging of tumors (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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