^{68}Ga-Labeled 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid-Tyr-cyclo(DAB-Arg-cyclo(Cys-Phe-D-Trp-Lys-Thr-Cys))

^{68}Ga-AM3

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Chemical name:	^{68}Ga-Labeled 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid-Tyr-cyclo(DAB-Arg-cyclo(Cys-Phe-D-Trp-Lys-Thr-Cys))	
Abbreviated name:	^{68}Ga-AM3	
Synonym:		
Agent Category:	Peptides	
Target:	Somatostatin receptors (SSTRs)	
Target Category:	Receptors	
Method of detection:	Positron emission tomography (PET)	
Source of signal / contrast:	^{68}Ga	
Activation:	No	
Studies:	• *In vitro* • Rodents	For structures of octreotide analogues, click on PubChem.

Background

[PubMed]

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The ^{68}Ga-labeled 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA)-Tyr-cyclo(DAB-Arg-cyclo(Cys-Phe-D-Trp-Lys-Thr-Cys)), abbreviated as ^{68}Ga-AM3, is a somatostatin (SST)-based, bicyclic peptide conjugate developed by Fani et al. for SST receptor (SSTR)–targeted imaging of neuroendocrine tumors (1).

The human SSTR family is a group of G-protein–coupled receptors with five members (SSTR1–SSTR5). All receptor members have seven α-helical transmembrane domains and possess a highly conserved sequence motif (YANSCANPI/VLY) in the seventh topology, which serves as a signature sequence for this family (2-4). Overall, there is 39%–57% sequence identity among the members, with the highest homology between SSTR1 and SSTR4, and among SSTR2, SSTR3, and SSTR5, respectively. The two groups of receptors also differ in their interactions with SST and its analogs (3-6). SSTR2, SSTR3, and SSTR5 have a high affinity for octreotide and seglitide, whereas SSTR1 and SSTR4 exhibit a very low affinity for them. With the exception of SSTR2, the precise contributions of other members remain to be elucidated. This is largely due to the lack of highly selective ligands and the co-expression of different members in single cells. SSTRs are distributed widely in cells both in the nervous system and periphery, and they have been shown to be overexpressed in a large number of malignancies, with particularly high density in neuroendocrine tumors (2, 7, 8).

As the targets of SST radiopharmaceuticals, SSTRs are of considerable clinical relevance for tumor imaging and radionuclide therapy (2, 9, 10). Because the native SST has a very short biological half-life (<2 min), various analogs have been synthesized, including a group of bicyclic peptides. Bicyclic peptides were first synthesized to understand the bioactive conformation and pharmacophoric amino acid sequence, and to increase the metabolic stability of the natural peptide SST-14 (also known as somatotropin release-inhibiting factor (SRIF)-14) by increasing the rigidity of bicyclic peptides (11, 12). Thereafter, various modified bicyclic peptides have been synthesized and have exhibited higher potency and longer duration of biological activity than SRIF-14 (1, 11, 12). As seen in the structure of the lead peptide cyclo(Aha,cyclo(Cys-Phe-D-Trp-Lys-Thr-Cys)), conformational constraints are introduced by head-to-tail coupling of a 16-atom ring with 7-aminoheptanoic acid (Aha) to the N- and C-terminally amino acid–deleted octreotide. To identify metabolically stable pansomatostatin analogs, a group of investigators from Switzerland have generated a set of bicyclic peptides by forming a second 16-atom ring with Arg and γ-aminobutyric acid (GABA) while keeping the octreotide 20-atom sequence as an inner circle (13-15). These modifications led to the development of another lead peptide, cyclo(Arg-cyclo(Cys-Phe-D-Trp-Lys-Thr-Cys)-GABA), which is named HR3005 (1). Arg has been shown to be important for broad binding to SSTR subtypes, and GABA can be exchanged with diaminobutyric acid (DAB) for chelator coupling. Based on the HR3005 sequence, four bicyclic analogs have been further synthesized by Fani et al. from the same group (1). The influence of the conformational constraints on receptor binding profiles and the pharmacokinetics of the respective radiopeptides have been investigated. Studies by Fani et al. have shown that the high rigidity of these bicyclic radiopeptides leads to agonistic ligands with good affinity for all five SSTRs (1). The pharmacokinetic data of the DOTA-conjugated bicyclic peptide AM3

(DOTA-Tyr-cyclo(DAB-Arg-cyclo(Cys-Phe-D-Trp-Lys-Thr-Cys))) make it an excellent candidate for use as an imaging radiotracer (1).

Related Resource Links:

- MICAD chapters on SSTR imaging
- Gene information on SSTRs
- Articles on SSTRs in Online Mendelian Inheritance in Man (OMIM)
- Somatostatin analogs in PubChem
- Bioassays of SSTRs in PubChem
- SSTR-related clinical trials in ClinicalTrial.gov

Synthesis

[PubMed]

Fani et al. synthesized AM3 on the basis of the lead peptide of HR3005 (1). The GABA in HR3005 was replaced with DAB, followed by coupling of the peptide with DOTA through Tyr as a spacer. The ^{68}Ga-labeled AM3 (^{68}Ga-AM3) was prepared according to the method of Zhernosekov et al. (16). The radiotracer solution was prepared by dilution with 0.9% NaCl containing 0.1% bovine serum albumin. The overall yield was ~30%. The ^{68}Ga-labeling yield was >97% with a specific activity of 35 GBq/μmol (0.95 Ci/μmol). The radiochemical purity of ^{68}Ga-AM3 was >95%.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Fani et al. characterized AM3 and ^{68}Ga-AM3 for their binding affinity, cell internalization, and effect on intracellular Ca^{2+} release (1).

The binding profile of AM3 was determined with receptor autoradiography. The cell membrane pellets were prepared from human embryonic kidney (HEK) cells expressing different subtypes of SSTRs (17). The binding affinity was expressed as 50% inhibitory concentration (IC_{50}; $n > 3$). The lead peptide HR3005 showed high affinity (comparable to SRIF-28) for SSTR2 (2.4 ± 0.6 nM) and SSTR3 (3.4 ± 0.8 nM), and moderate affinity for SSTR5 (14.5 ± 4.5 nM), SSTR1 (34 ± 10 nM), and SSTR4 (68 ± 22 nM), conferring almost pansomatostatin potency to this peptide. Replacement of GABA with DAB in the HR3005 did not change the affinity profile, whereas DOTA-coupling led to marked loss of affinity for all SSTR subtypes. By introducing Tyr as a spacer between DOTA and the peptide, the affinity of generated AM3 recovered to levels comparable to those of HR3005. The IC_{50} values of AM3 were 119 ± 6 nM for SSTR1, 2.3 ± 0.2 nM for SSTR2, 4.0 ± 0.03 nM for SSTR3, 97 ± 21 nM for SSTR4, and 27 ± 1 nM for SSTR5. The binding profile of ^{68}Ga-AM3 was not examined.

The immunofluorescence microscopy–based internalization assay for SSTR2 and SSTR3 with HEK-SSTR2 and HEK-SSTR3 cells showed that AM3 elicited a pronounced

relocation of SSTR2 and SSTR3 from the plasma membrane to the cytoplasm at 30 min after it was applied to the cells, an effect similar to that of the control, SRIF-28.

The stimulating effect of AM3 on the intracellular Ca^{2+} release was tested with HEK cells expressing SSTR2, SSTR3, and SSTR5. Intracellular Ca^{2+} release is a part of the signaling pathway regulated by native SST. On SSTR3 and SSTR5, no signal was found. On SSTR2, a large, concentration-dependent increase of the signal was observed. This indicates that AM3 is an agonist because of its stimulating effect on the Ca^{2+} release and high potencies similar to those of SRIF-14. The agonist-elicited response in terms of the half maximal effective concentration value was 21.5 ± 3.5 nM.

The internalization rate and efflux of ^{177}Lu-AM3 were studied in HEK-SSTR2 and HEK-SSTR3 cells (for details, refer to the chapter on ^{177}Lu-AM3 in MICAD). Similar experiments were not performed for ^{68}Ga-AM3. ^{177}Lu-AM3 was extremely stable in human blood and serum at 37°C. However, the stability of ^{68}Ga-AM3 was not tested.

Animal Studies

Rodents

[PubMed]

Five-week-old athymic female Swiss nude mice with HEK-SSTR2 tumors (n = 4–7 mice/group) were used for biodistribution studies after injection of ^{68}Ga-AM3 (100 µl/10 pmol per 0.4 MBq (0.011 mCi)) into the tail vein (1). Nonspecific uptake was determined with co-injection of 2,000-fold excess DOTANOC (18, 19). One group of mice was pre-injected with lysine 10 min before the injection of ^{68}Ga-AM3 to study kidney blocking. The results were expressed as percentage of injected dose per gram of tissue (% ID/g).

The biodistribution profile of ^{68}Ga-AM3 at early times was similar to that of ^{177}Lu-AM3, showing fast blood clearance, fast nontarget clearance, and high receptor-mediated uptake in the tumors. At 1 h after injection, the SSTR2 tumors accumulated the highest ^{68}Ga-AM3, with an uptake value of 11.92 ± 1.22% ID/g and a tumor/kidney ratio of 1.2. Blocking experiments with DOTANOC (high affinities for SSTR2, SSTR3, and SSTR5) confirmed the receptor-mediated uptake. Pre-injection of lysine (20 mg/100 µl) resulted in ~50% reduction of the kidney uptake with no significant impact on the tumor uptake. For PET/CT studies, the mice bearing HEK-SSTR2 tumors were injected with ^{68}Ga-AM3, including a group preinjected with lysine and a group co-injected with DOTANOC (1). The mice were euthanized at 1 h after injection and scanned for 60 min using a routine combined PET/CT scanner. The images clearly showed high tumor and kidney uptake but negligible background. Preinjection of lysine resulted in a significant reduction of the kidney uptake. The specific uptake of ^{68}Ga-AM3 was also confirmed using DOTANOC as a blocking agent, with which only the kidneys were visualized. The imaging study results were consistent with the biodistribution data.

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.

References

1. Fani M., Mueller A., Tamma M.L., Nicolas G., Rink H.R., Cescato R., Reubi J.C., Maecke H.R. *Radiolabeled bicyclic somatostatin-based analogs: a novel class of potential radiotracers for SPECT/PET of neuroendocrine tumors.* J Nucl Med. 2010;51(11):1771–9. PubMed PMID: 20956465.
2. Froidevaux S., Eberle A.N. *Somatostatin analogs and radiopeptides in cancer therapy.* Biopolymers. 2002;66(3):161–83. PubMed PMID: 12385036.
3. Tulipano G., Schulz S. *Novel insights in somatostatin receptor physiology.* Eur J Endocrinol. 2007;156 Suppl 1:S3–11. PubMed PMID: 17413186.
4. van der Hoek J., Hofland L.J., Lamberts S.W. *Novel subtype specific and universal somatostatin analogues: clinical potential and pitfalls.* Curr Pharm Des. 2005;11(12): 1573–92. PubMed PMID: 15892663.
5. Lesche S., Lehmann D., Nagel F., Schmid H.A., Schulz S. *Differential effects of octreotide and pasireotide on somatostatin receptor internalization and trafficking in vitro.* J Clin Endocrinol Metab. 2009;94(2):654–61. PubMed PMID: 19001514.
6. Yim C.B., Dijkgraaf I., Merkx R., Versluis C., Eek A., Mulder G.E., Rijkers D.T., Boerman O.C., Liskamp R.M. *Synthesis of DOTA-conjugated multimeric [Tyr3]octreotide peptides via a combination of Cu(I)-catalyzed "click" cycloaddition and thio acid/sulfonyl azide "sulfo-click" amidation and their in vivo evaluation.* J Med Chem. 2010;53(10):3944–53. PubMed PMID: 20411957.
7. Strowski M.Z., Blake A.D. *Function and expression of somatostatin receptors of the endocrine pancreas.* Mol Cell Endocrinol. 2008;286(1-2):169–79. PubMed PMID: 18375050.
8. Kwekkeboom D.J., Kam B.L., van Essen M., Teunissen J.J., van Eijck C.H., Valkema R., de Jong M., de Herder W.W., Krenning E.P. *Somatostatin-receptor-based imaging and therapy of gastroenteropancreatic neuroendocrine tumors.* Endocr Relat Cancer. 2010;17(1):R53–73. PubMed PMID: 19995807.

9. Bal C.S., Gupta S.K., Zaknun J.J. *Radiolabeled somatostatin analogs for radionuclide imaging and therapy in patients with gastroenteropancreatic neuroendocrine tumors.* Trop Gastroenterol. 2010;31(2):87–95. PubMed PMID: 20862981.
10. de Jong M., Breeman W.A., Kwekkeboom D.J., Valkema R., Krenning E.P. *Tumor imaging and therapy using radiolabeled somatostatin analogues.* Acc Chem Res. 2009;42(7):873–80. PubMed PMID: 19445476.
11. Veber D.F., Holly F.W., Paleveda W.J., Nutt R.F., Bergstrand S.J., Torchiana M., Glitzer M.S., Saperstein R., Hirschmann R. *Conformationally restricted bicyclic analogs of somatostatin.* Proc Natl Acad Sci U S A. 1978;75(6):2636–40. PubMed PMID: 208068.
12. Veber D.F., Freidlinger R.M., Perlow D.S., Paleveda W.J. Jr, Holly F.W., Strachan R.G., Nutt R.F., Arison B.H., Homnick C., Randall W.C., Glitzer M.S., Saperstein R., Hirschmann R. *A potent cyclic hexapeptide analogue of somatostatin.* Nature. 1981;292(5818):55–8. PubMed PMID: 6116194.
13. Ginj M., Hinni K., Tschumi S., Schulz S., Maecke H.R. *Trifunctional somatostatin-based derivatives designed for targeted radiotherapy using auger electron emitters.* J Nucl Med. 2005;46(12):2097–103. PubMed PMID: 16330576.
14. Ginj M., Schmitt J.S., Chen J., Waser B., Reubi J.C., de Jong M., Schulz S., Maecke H.R. *Design, synthesis, and biological evaluation of somatostatin-based radiopeptides.* Chem Biol. 2006;13(10):1081–90. PubMed PMID: 17052612.
15. Ginj M., Zhang H., Waser B., Cescato R., Wild D., Wang X., Erchegyi J., Rivier J., Macke H.R., Reubi J.C. *Radiolabeled somatostatin receptor antagonists are preferable to agonists for in vivo peptide receptor targeting of tumors.* Proc Natl Acad Sci U S A. 2006;103(44):16436–41. PubMed PMID: 17056720.
16. Zhernosekov K.P., Filosofov D.V., Baum R.P., Aschoff P., Bihl H., Razbash A.A., Jahn M., Jennewein M., Rosch F. *Processing of generator-produced 68Ga for medical application.* J Nucl Med. 2007;48(10):1741–8. PubMed PMID: 17873136.
17. Reubi J.C., Schar J.C., Waser B., Wenger S., Heppeler A., Schmitt J.S., Macke H.R. *Affinity profiles for human somatostatin receptor subtypes SST1-SST5 of somatostatin radiotracers selected for scintigraphic and radiotherapeutic use.* Eur J Nucl Med. 2000;27(3):273–82. PubMed PMID: 10774879.
18. Wild D., Schmitt J.S., Ginj M., Macke H.R., Bernard B.F., Krenning E., De Jong M., Wenger S., Reubi J.C. *DOTA-NOC, a high-affinity ligand of somatostatin receptor subtypes 2, 3 and 5 for labelling with various radiometals.* Eur J Nucl Med Mol Imaging. 2003;30(10):1338–47. PubMed PMID: 12937948.
19. Wild D., Macke H.R., Waser B., Reubi J.C., Ginj M., Rasch H., Muller-Brand J., Hofmann M. *68Ga-DOTANOC: a first compound for PET imaging with high affinity for somatostatin receptor subtypes 2 and 5.* Eur J Nucl Med Mol Imaging. 2005;32(6): 724. PubMed PMID: 15551131.