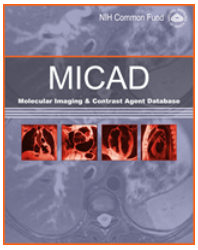
U.S. National Library of Medicine
National Center for Biotechnology Information

NLM Citation: Chopra A. ^{64}Cu-Labeled 1,1′-{1,4-phenylenebis(methylene)}-bis{1,4,8,11-tetraaza-cyclotetradecane}. 2009 Jun 8 [Updated 2009 Jul 7]. In: Molecular Imaging and Contrast Agent Database (MICAD) [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2004-2013.
Bookshelf URL: https://www.ncbi.nlm.nih.gov/books/



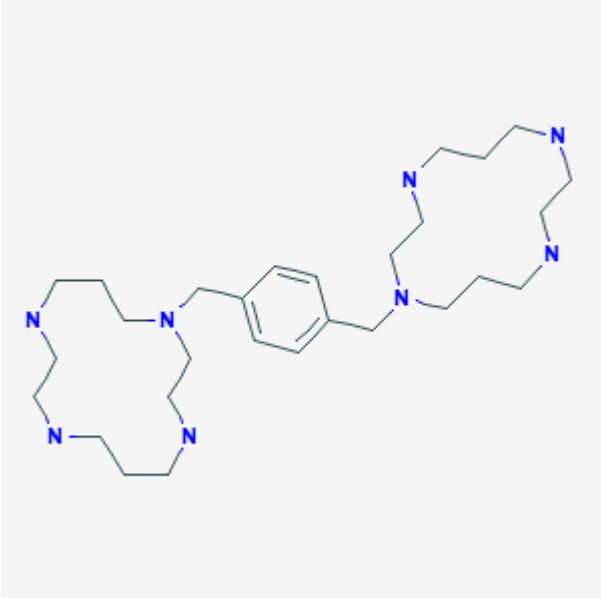
^{64}Cu-Labeled 1,1′-{1,4-phenylenebis(methylene)}-bis{1,4,8,11-tetraaza-cyclotetradecane}

[^{64}Cu]-AMD3100

Arvind Chopra, PhD[1]

Created: June 8, 2009; Updated: July 7, 2009.

Chemical name:	^{64}Cu-Labeled 1,1′-{1,4-phenylenebis(methylene)}-bis{1,4,8,11-tetraaza-cyclotetradecane}
Abbreviated name:	[^{64}Cu]-AMD3100
Synonym:	[^{64}Cu]-4
Agent Category:	Ligand
Target:	Chemokine receptor 4 (CXCR4)
Target Category:	Receptor
Method of detection:	Positron emission tomography (PET)
Source of signal / contrast:	^{64}Cu
Activation:	No
Studies:	• *In vitro* • Rodents



Click on the above structure of AMD3100 for additional information regarding the compound in PubChem.

Background

[PubMed]

The chemokine receptor 4 (CXCR4) and its ligand, the stromal cell–derived factor-1 (SDF-1 or CXCL12), are known to play a major role in the migration of progenitor cells during embryonic development of the central nervous, cardiovascular, and the hematopoietic systems (1, 2). In addition, this receptor–ligand pair has a function in the development, progression, and spread of various cancers (3), and the CXCR4 acts as a co-

Author Affiliation: 1 National Center for Biotechnology Information, NLM, NIH, Bethesda, MD 20894; Email: micad@ncbi.nlm.nih.gov.

receptor to facilitate entry of the human immunodeficiency virus (HIV) into $CD4^+$ cells (4). It has also been suggested that CXCR4 and SDF-1 participate in the pathogenesis of neurodegenerative and inflammatory conditions (5). Because of its role in the development of cancer and HIV infections, a variety of CXCR4 inhibitors, including 1,1'-{1,4-phenylenebis(methylene)}-bis{1,4,8,11-tetraaza-cyclotetradecane} (AMD3100), have been evaluated for the treatment of these ailments (6, 7).

Some imaging studies have also been performed on CXCR4 (8, 9). Technetium (^{99m}Tc)-labeled SDF-1 was shown to be a suitable probe to quantify CXCR4 levels under *in vivo* conditions and could be used to determine changes in CXCR4 expression in different tissue under various pathological and physiological conditions (8). An indium (^{111}In)-labeled CXCR4 antagonist, [^{111}In]-Ac-TZ14011, was developed for the imaging of CXCR4 expression in xenograft tumors in mice, and Hanaoka et al. used single-photon emission computed tomography to investigate the biodistribution of [^{111}In]-Ac-TZ14011 in mice (9). However, the labeled compound showed a low accumulation in the tumors (<1% of the injected dose/gram tissue (% ID/g)), and a high accumulation was reported in the liver (19.3% ID/g), kidneys (29.5% ID/g), and the spleen (5.83% ID/g). These results indicated that [^{111}In]-Ac-TZ14011 was suitable for the imaging of CXCR4, although it lacked tumor specificity and the high accumulation of radioactivity in the liver and kidneys showed that this radiochemical was not suitable for the imaging of tumors in these organs.

In an effort to develop a superior CXCR4 imaging agent, AMD3100 was labeled with radioactive copper (^{64}Cu) to produce [^{64}Cu]-AMD3100, and it was evaluated as a positron emission tomography imaging agent by Jacobson et al. (10). The investigators also studied the biodistribution of [^{64}Cu]-AMD3100 in normal mice.

Synthesis

[PubMed]

The synthesis of AMD3100 was described by Jacobson et al. (10). AMD3100 was labeled with ^{64}Cu by the addition of ^{64}Cu-acetate to a solution of AMD3100 dissolved in 0.4 M ammonium acetate (pH 5.5) followed by stirring for 1 h at room temperature (10). Radiochemical purity of [^{64}Cu]-AMD3100 was determined with thin-layer chromatography (TLC). The Rf of labeled AMD3100 was reported to be ~0.1, and that of free ^{64}Cu was ~0.6, as determined with TLC. Although the radiochemical labeling yield was not stated the radiochemical yield and purity of [^{64}Cu]-AMD3100 were reported to be 100%, respectively, under these analytical conditions. The radiotracer had a specific activity of 417 TBq/mmol (11,280 Ci/mmol).

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Using a cell binding assay the 50% inhibitory concentration (IC_{50}) of [^{64}Cu]-AMD3100 for Jurkat cells (which were shown to have a high expression of the CXCR4 (10)) was reported to be 62.7 μM (10). A similar study by the investigators showed that the IC_{50} of [^{64}Cu]-AMD3100 for mouse splenocytes was 46.9 μM.

To confirm that no changes had occurred in the biological activity (CXCR4 inhibition potential) of Cu-AMD3100, due to the incorporation of Cu, the ability of Cu-AMD3100 to inhibit Jurkat cell migration toward stromal cell-derived factor-1 in a transwell migration assay was compared to that of AMD3100 under the same experimental conditions (10). The IC_{50}s of AMD3100 and Cu-AMD3100 required to inhibit the Jurkat cell migration were reported to be 27.4 and 75.4 nM, respectively.

The exact reasons for the discrepancy observed between the IC_{50} values reported for the binding and the transwell migration assays were not provided by the investigators.

Animal Studies

Rodents

[PubMed]

Jacobson et al. studied the biodistribution of [^{64}Cu]-AMD3100 in six groups of normal C57BL/6 mice (n = 5 animals/group) (10). Three groups of animals received intravenous injections of [^{64}Cu]-AMD3100 and were euthanized 1, 2, and 6 h later. Groups four and five were co-injected with [^{64}Cu]-AMD3100 and excess (50 µg) unlabeled AMD3100. These animals were euthanized 2 and 6 h after injection. The sixth group was injected with [^{64}Cu]-AMD3100 in the presence of SDF-1 to determine specificity of the radiopharmaceutical for binding to CXCR4. These animals were euthanized 2 h after treatment. The thymus, liver, spleen, intestine, femoral bone marrow, muscle, blood, kidneys, and lymph nodes were removed from all the animals to determine accumulation of radioactivity in the various organs of the groups treated with [^{64}Cu]-AMD3100,. In animals treated with [^{64}Cu]-AMD3100 alone, the maximum accumulation of radioactivity was observed in the liver (~40–55% ID/g at the different time points), followed by spleen and bone marrow (~12–15% ID/g at the various time points), lymph nodes (~10% ID/g at all the time points), and the kidneys (~7% ID/g at the predetermined time points). Animals co-injected with the radiopharmaceutical and the unlabeled AMD3100 showed a reduced accumulation of radioactivity in the liver, bone marrow, and the lymph nodes 6 h after injection, which indicated that the radioactivity bound specifically to the CXCR4 in these organs (for details, please see Figure 7 in Jacobson et al. (10)).

Imaging of animals (n = 5 mice) injected with [^{64}Cu]-AMD3100 alone revealed that the label accumulated mainly in the liver, kidneys, and spleen as observed during the biodistribution studies described above (10). Blocking of [^{64}Cu]-AMD3100 uptake with excess unlabeled AMD3100 (n = 5 mice) resulted in a reduced accumulation of radioactivity in these organs. During the same time, imaging showed an accumulation of radioactivity in the kidneys. To confirm that the accumulated radioactivity in the animals (n = 5 mice) was not due to free ^{64}Cu, the mice were treated with the free ^{64}Cu isotope and scanned 2 h later. In these animals the radioactivity accumulated mainly in the liver and intestines, but not in the spleen and lymph nodes, which are known to contain a high level of CXCR4 receptors. This indicated that free ^{64}Cu accumulated primarily in organs involved in the excretion of the labeled metal and that the radioactivity observed in the spleen and the lymph nodes during the biodistribution and imaging studies was from the labeled AMD3100.

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

[Disclaimer]

No information is currently available.

NIH Support

Intramural Research Programs of the National Institute of Biomedical Imaging and Bioengineering and the National Institute of Allergy and Infectious Diseases.

References

1. Juarez J., Bendall L. *SDF-1 and CXCR4 in normal and malignant hematopoiesis.* . Histol Histopathol. 2004;19(1):299–309. PubMed PMID: 14702198.
2. Miller R.J., Banisadr G., Bhattacharyya B.J. *CXCR4 signaling in the regulation of stem cell migration and development.* . J Neuroimmunol. 2008;198(1-2):31–8. PubMed PMID: 18508132.
3. Rubin J.B. *Chemokine signaling in cancer: one hump or two?* . Semin Cancer Biol. 2009;19(2):116–22. PubMed PMID: 18992347.
4. Alkhatib G. *The biology of CCR5 and CXCR4.* . Curr Opin HIV AIDS. 2009;4(2):96–103. PubMed PMID: 19339947.
5. Mocchetti I., Bachis A., Masliah E. *Chemokine receptors and neurotrophic factors: potential therapy against aids dementia?* . J Neurosci Res. 2008;86(2):243–55. PubMed PMID: 17847079.
6. Kuritzkes D.R. *HIV-1 entry inhibitors: an overview.* . Curr Opin HIV AIDS. 2009;4(2):82–7. PubMed PMID: 19339945.
7. Otsuka S., Bebb G. *The CXCR4/SDF-1 chemokine receptor axis: a new target therapeutic for non-small cell lung cancer.* . J Thorac Oncol. 2008;3(12):1379–83. PubMed PMID: 19057260.
8. Misra P., Lebeche D., Ly H., Schwarzkopf M., Diaz G., Hajjar R.J., Schecter A.D., Frangioni J.V. *Quantitation of CXCR4 expression in myocardial infarction using 99mTc-labeled SDF-1alpha.* . J Nucl Med. 2008;49(6):963–9. PubMed PMID: 18483105.
9. Hanaoka H., Mukai T., Tamamura H., Mori T., Ishino S., Ogawa K., Iida Y., Doi R., Fujii N., Saji H. *Development of a 111In-labeled peptide derivative targeting a chemokine receptor, CXCR4, for imaging tumors.* . Nucl Med Biol. 2006;33(4):489–94. PubMed PMID: 16720240.
10. Jacobson O., Weiss I.D., Szajek L., Farber J.M., Kiesewetter D.O. *64Cu-AMD3100--a novel imaging agent for targeting chemokine receptor CXCR4.* . Bioorg Med Chem. 2009;17(4):1486–93. PubMed PMID: 19188071.