

# Preparation and Quality Control of $^{111}\text{In}$ -Plerixafor for Chemokine Receptor CXCR4

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Received: 1 November 2014; Revised: 12 May 2015; Accepted: 22 May 2015

**Abstract:** Developing CXCR4 receptor imaging agents is of great importance for oncological imaging. In this work, N, N'-[1, 4-Phenylenebis(methylene)]bis-1,4,8,11-tetraazacyclotetradecane was labeled using high purity [ $^{111}\text{In}$ ]InCl<sub>3</sub> in 30-60 min at 60°C in acetate buffer with acceptable yield and radiochemical purity (>97% ITLC, >95% HPLC, specific activity: 120-200 GBq/mmol). Radiolabeled complex of AMD-3100 ([ $^{111}\text{In}$ ]-AMD3100) showed good stability at room temperature and physiologic conditions in human serum for 24 h. Log P for the complex was determined (-1.18) to be consistent with a water soluble/ionic complex.

The biodistribution of the radiolabeled complex of plerixafor in vital organs of rats was determined up to 48 h demonstrating kidneys as the major route of excretion. Considering lungs, spleen and liver as the CXCR4 rich target organs, the best target:non target ratios (for spleen:blood, lung:blood and liver:blood) were obtained 24 h post injection (5.11, 2.08 and 10.63 respectively).

**Keywords:** AMD3100, biodistribution, CXCR4, In-111, plerixafor, radiolabeling.

## INTRODUCTION

Many antagonist compounds have been developed against the chemokine receptor subtype receptor (CXCR4) including peptidic and non peptidic ligands with different modes of action [1]. CXCR4 is overexpressed in many human solid tumors and has attracted interest as a pharmacological target for cancer treatment as well as diagnosis. A list of relevant tumors includes prostate cancer, breast cancer, melanoma, B-cell lymphoma, cervical adenocarcinoma neuroblastoma, and gliomas [2], involved in tumor growth, angiogenesis, cancer cell migration, and metastasis.

CXCR4 interacting ligands might constitute promising targeting molecules for specific imaging studies to demonstrate the initiation of proliferation and/or promote survival of various metastatic malignant cells *in vivo* and suggesting CXCR4 antagonist such as; N, N'-[1, 4-Phenylenebis

(methylene)]bis-1,4,8,11-tetraazacyclotetradecane (Plerixafor; AMD3100), Fig. (1), to be used in developing imaging agents.

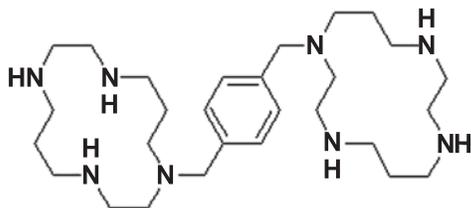
Interesting synergistic binding affinity of metal-AMD3100 complexes compared to parent AMD3100 ligand has been observed, such as copper complex (increased by 6-fold) [3] and binuclear complexes of Zn(II), Cu(II) and Ni(II)-plerixafor. Interestingly, the Zn(II)-plerixafor complex (carrying overall +4 charge), divulged higher specificity and reduced toxicity *in vitro* compared to the free ligand [4].

Various CXCR4 imaging compounds based on AMD-3100, using single photon emission tomography (SPECT) and positron emission tomography (PET) including Tc-99m [5], Cu-64 [6, 7], Zn-62/Cu-62 [8] and Ga-67 [9] have been reported, however the attempt to develop SPECT/PET imaging agents is of great importance in radiopharmaceutical sciences. The pharmacologic properties of porphyrins develop the idea of possible tumor imaging agent using PET by incorporating Ga-68 into a suitable porphyrin ligand [10].

The In-111 labeled peptidic CXCR4 antagonists have been developed [11] and used in the imaging of mammary intraepithelial neoplastic outgrowth bearing mice without

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significant results. However, the major aspect of tracer development for this receptor has been focused on small cyclam containing molecules such as AMD-3100.



**Fig. (1).** Chemical structure of AMD3100.

Due to known and approved protocols for In-111 imaging in nuclear medicine development of an In-111 labeled complex with repeated and prolonged imaging studies and follow up in possible human patients would be interesting. Most of tumor cell uptake and localizations need longer circulation times compared to that of reported short half-life positron emitter AMD3100 analogs.

In this investigation, by using a cyclotron-produced radionuclide (In-111, half-life: 67 h), an In-111 AMD-3100 complex for long term imaging of CXCR4 receptors *in vivo* compared to Ga-68, Zn-62 analogs using SPECT was developed and radiolabeling, quality control, partition coefficient determination ( $\log P$ ), biological distribution studies and stability tests of [ $^{111}\text{In}$ ]-AMD3100 in wild-type rats are reported.

## MATERIALS AND METHODS

AMD3100 hydrochloride and other chemicals were purchased from the Sigma-Aldrich Chemical Co. (Germany); and the ion-exchange resins from Bio-Rad Laboratories (Canada). HPLC used to determine the specific activity of the labeled compound, was performed by a Shimadzu LC-10AT, equipped with two detector systems, UV-visible (Shimadzu) using Whatman Partisphere C-18 column 250×4.6 mm and flow scintillation analyzer (Packard-150TR), NJ (USA). Instant thin layer chromatography (ITLC) was operated on aluminum-backed silica gel (F 254, Schleicher & Schuell®, Germany) or Whatman No. 2 papers using a thin layer chromatography scanner, Bioscan AR2000, Bioscan Europe Ltd. (France) [9]. Normal saline and other solvents, used for labeling were of high purity and had been filtered through 0.22  $\mu\text{m}$  Cativex filters. Biodistribution data were acquired by counting normal saline washed tissues after weighting on a Canberra™ high purity germanium (HPGe) detector (model GC1020-7500SL) [9]. All calculations and tissue counting were based on the 172 keV peak. Animal studies were performed in accordance with the United Kingdom Biological Council's Guidelines on the Use of Living Animals in Scientific Investigations, 2nd Ed.

### Preparation of [ $^{111}\text{In}$ ]-AMD3100

Because of its stability and cell avidity of metal maltol complexes, [ $^{111}\text{In}$ ]-maltolate can be an exciting cell imaging agent, but needs more bio distribution studies in various cells as well as investigative procedures [12]. The nuclear reaction  $^{112}\text{Cd}$  (p, 2n)  $^{111}\text{In}$  was

used for the production of  $^{111}\text{In}$ . Cd-112 was electroplated on the surface of a copper target according to the reported method [13].  $^{111}\text{In}$ -Indium chloride produced by proton bombardment of cadmium-112 in 22 MeV at the 30 MeV cyclotron (from IBA), with a current of 100  $\mu\text{A}$  for 48 min (80  $\mu\text{Ah}$ ). The acidic solution (100  $\mu\text{L}$ ) of [ $^{111}\text{In}$ ]InCl<sub>3</sub> (3-5 mCi) in a borosilicate vial was heated to evaporate the solvent using a flow of N<sub>2</sub> gas at 50-60°C, followed by the addition of ammonium acetate solution (200  $\mu\text{L}$ , 0.5 M). 150  $\mu\text{L}$  of plerixafor dissolved in acetate buffer pH=5 (5mg/ml, about 0.9-1  $\mu\text{moles}$ ) was added to the indium-containing vial and the stirred mixture was heated to 60 °C for 1h. The active solution was checked for radiochemical purity using Whatman No. 2 paper and developed in mobile phase mixture, methanol and 10% ammonium acetate 1:1. HPLC was performed on the final preparation using a mixture of acetonitrile:water 2:3 (v/v) as the eluent by means of RP column Whatman Partisphere C<sub>18</sub> 4.6×250 mm.

### Determination of Partition Coefficient

In order to determine partition coefficient ( $\log P$ ) of [ $^{111}\text{In}$ ]-plerixafor, the mixture of 1 ml of isotonic acetate-buffered saline (pH=7) and 1 ml of 1-octanol containing approximately 3.7 MBq of [ $^{111}\text{In}$ ]-AMD3100 at 37°C was vortexed 1 min and left 5 min. Following centrifugation at >1500g for 3 min, the organic and aqueous phases were sampled and counted in a well-type counter. A 500  $\mu\text{L}$  sample of the octanol phase from this experiment was shaken again 2 to 3 times with fresh buffer samples. The reported  $\log P$  values are the average of the second and third extractions from three to four independent measurements [10].

### Stability Tests of [ $^{111}\text{In}$ ]-AMD3100

The stability of the labeled compound was checked according to the conventional ITLC method using a sample of [ $^{111}\text{In}$ ]-AMD3100 (37 MBq) stored at room temperature for up to 72 h. For serum stability, final solution (400  $\mu\text{Ci}$ , 100  $\mu\text{L}$ ) was incubated in physiologic conditions in human serum (300  $\mu\text{L}$ ). Every 2 h to portion of the mixture (50  $\mu\text{L}$ ), trichloroacetic acid (10%, 100  $\mu\text{L}$ ) was added and the sample was centrifuged at 3000 rpm for 3 min followed by decanting the supernatant from the debris. The stability of the labeled compound was confirmed by performing frequent ITLC analysis of supernatant using the mentioned ITLC system.

### Biodistribution of [ $^{111}\text{In}$ ]-AMD3100 in Rats

The distribution of the radiolabeled complex among tissues was determined for wild-type rats. The total amount of radioactivity injected into each animal was measured by counting the 1-ml syringe before and after injection in a dose calibrator with fixed geometry [9]. For blocking tests co-injection of the tracer with cold unlabeled AMD-3100 was performed followed by scarification in 2 and 4 h post injection. The rats were sacrificed using the animal care protocols at selected times after injection, the tissues were washed with normal saline after weighting and their specific activities were determined with an HPGe detector equipped

with a sample holder device as percent of injected dose per gram of tissues [10].

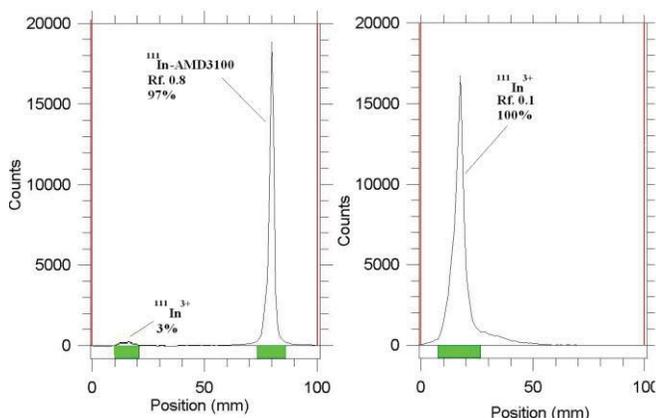
### Imaging of [ $^{111}\text{In}$ ]-AMD3100 in Rats

Rats injected with [ $^{111}\text{In}$ ]-AMD3100 were used for imaging. Images were taken at various time intervals after administration of the complex by a dual-head SPECT system. The mouse-to-high energy septa distance was 12 cm. The useful field of view (UFOV) was 540 mm $\times$ 400 mm. The spatial resolution was 10 mm FWHM at the central field of view (CFOV). 64 projections were acquired for 30 seconds per view with a 64 $\times$ 64 matrix [12].

## RESULTS AND DISCUSSION

### Radiolabeling

Due to NH polar functional groups participation in cyclam structure, labeling of AMD3100 with indium cation affects its chromatographic properties and the final [ $^{111}\text{In}$ ]-AMD3100 is more lipophilic. Thus a chromatographic system was used for the detection of the radiolabeled compound from the free indium cation. Using methanol and 10% ammonium acetate 1:1 mixture, indium cation remains at the origin of the paper as a single peak, while [ $^{111}\text{In}$ ]-AMD3100 migrates to higher  $R_f$  (0.8), Fig. (2). Although the nature of the plerixafor radiolabeled [10] complexes is reported to be mostly ionic as observed for Zn(II)-plerixafor complex with 4 cationic centers, in this case the complex still possesses higher lipophilicity compared to free cation.



**Fig. (2).** ITLC of [ $^{111}\text{In}$ ] $\text{InCl}_3$  (right) and [ $^{111}\text{In}$ ] $\text{InAMD3100}$  (left) in a methanol and 10% ammonium acetate 1:1 mixture as mobile phase on Whatman No.2 papers.

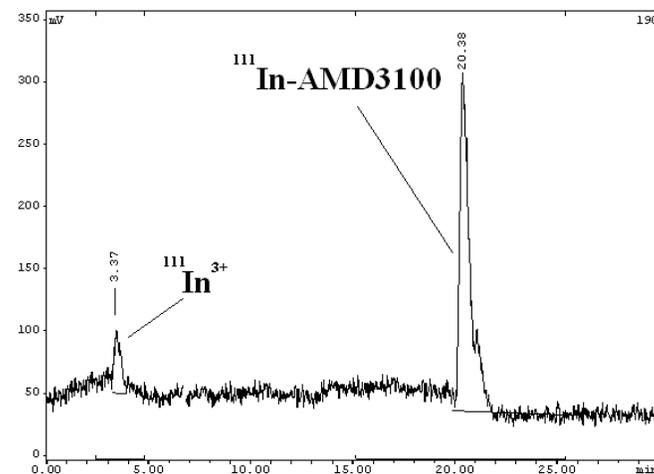
The ITLC studies confirmed the production of radiolabeled complex, HPLC studies corroborated the existence of radiolabeled species using both UV and scintillation detectors [12]. A fast-eluting compound at 3.37 min (scintillation detector) related to 3.2 min peak (UV detector) demonstrated a hydrophilic species related to  $\text{In}^{3+}$  cation. A late eluted compound at 20.38 min was related to radiolabeled complex with more than 95-97 radiochemical purity, Fig. (3).

The partition coefficient (log P) of the complex depended on the pH of the solution. Log P was -1.18 at the pH.7. The water solubility of the radio-complex leads to less non-specific uptakes in tissues including liver and fat and faster

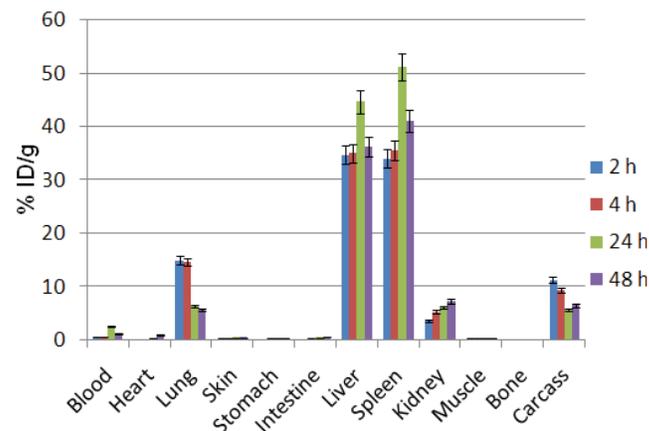
kidney wash-out. Incubation of [ $^{111}\text{In}$ ]-AMD3100 in human serum for 24 h at 37 $^\circ\text{C}$  showed no loss of  $^{111}\text{In}$  from the complex. The radiochemical purity of radiolabeled compound persisted >97% for 24 h under physiologic conditions as shown by ITLC method.

### [ $^{111}\text{In}$ ]- $\text{InCl}_3$ biodistribution in wild-type rats' tissues:

For better comparison a biodistribution study was performed for free  $\text{In}^{3+}$ . As reported previously, indium cation almost mimics the ferric cation behavior and is rapidly removed from the circulation and accumulated in the liver (35-40 %) in the liver after 24 h also a major fraction is accumulated in the spleen and smaller part is excreted through the urine as a water soluble cation Fig. (4).



**Fig. (3).** HPLC chromatogram of [ $^{111}\text{In}$ ]-AMD3100 solution on a reversed phase column using water + 0.1% TFA/acetonitrile+0.1% TFA/, 10:90.



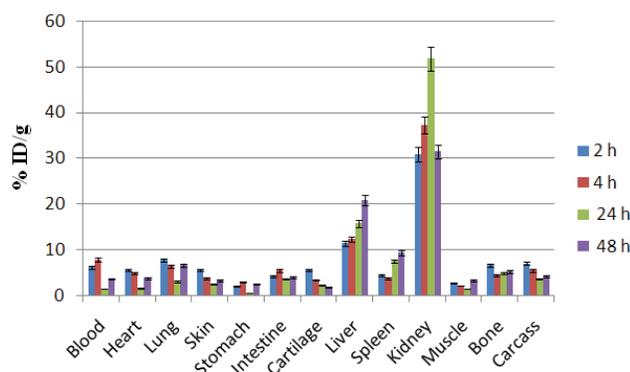
**Fig. (4).** Biodistribution of  $^{111}\text{In}$ - $\text{InCl}_3$  (1.85 MBq) in wild-type rats 2, 4 and 24 h after iv injection *via* tail vein (ID/g%: percentage of the injected dose per gram of tissue calculated based on the area under curve of 172 keV peak in gamma spectrum) (n=3).

### Biodistribution Studies of [ $^{111}\text{In}$ ]-AMD3100 in Rats

The animals were sacrificed by  $\text{CO}_2$  asphyxiation at selected times after injection [9]. The uptake of [ $^{111}\text{In}$ ]-AMD3100 complex in different organs or tissues was calculated as percentage of the injected dose per gram organs or tissues of rats (% ID/g) [9]. The radio-complex circulates in blood for at least 4 hours affording high bioavailability of

the complex. Kidney is a major site of excretion for the labeled compound especially up to 48 h post administration, Fig. (5).

This has already been shown in other radiolabeled analogs on rat animals for kidney uptake percentage, for instance 4.5 % for  $^{67}\text{Ga}$ -analog [9], 12% for Sm-153 analog [14] and 5% for Zn/Cu analog [8]. The possible hydrophilic/ionic nature of  $^{111}\text{In}$ -AMD3100 increased the kidney uptake however, due to receptor binding in kidney excretion is not a major cause of kidney uptake leading to the increase in kidney radioactivity content.



**Fig. (5).** Percentage of injected dose per gram (% ID/g) of  $^{111}\text{In}$ -AMD3100 in rats tissues at 2-48 h post-injection. post-injection based on 172 keV photopeak (n=3).

CXCR4 receptor is expressed in normal organs such as lungs, bone marrow, and liver and much less in other organs [15]. Biodistribution studies of  $^{111}\text{In}$ -AMD3100 in rats showed rapid washout of the tracer from the urinary tract. The stability of the  $^{111}\text{In}$ -plerixafor produced by *in vitro* methods does not allow the detachment of the radio cation into blood and other organs, thus kidneys are the most important excretion organs and possible critical organ in the dosimetry calculations [9]. Unlike  $\text{In}^{3+}$  cation, stomach, heart, bone, muscle, and skin do not demonstrate significant uptakes at all-time intervals.

Liver, spleen and kidney are the only significant uptake targets. From the data it can be suggested that  $^{111}\text{In}$ -plerixafor is excreted from and/or metabolized through the kidneys and hepatic metabolism respectively [9]. However, no metabolic study was performed to identify the natures of metabolite(s).

The high kidney uptake can cause extra dose to surrounding critical tissues including the gonads; this can be an impediment [9].

In a previous report using  $^{125}\text{I}$ -anti CXCR4, spleen and liver have been shown high accumulation, possibly due to the presence of CXCR4-containing blood cells [16]. With respect to this work, the major receptor rich organs can be considered liver and spleen. However, the high kidney uptake is a result of being the major excretion organ due to high water solubility of the complex, and not the receptor mediated uptake [9].

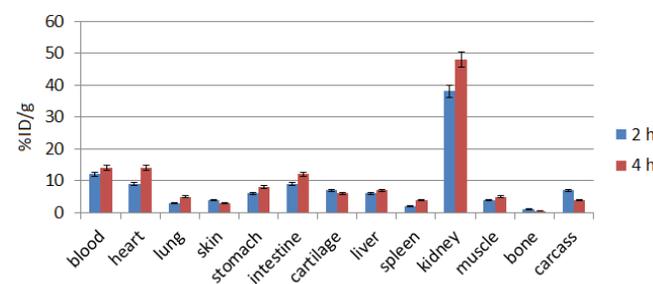
Table 1 demonstrates the ratios for highly expressed CXCR4 tissues including liver, spleen and lungs to blood as a possible imaging agent at various time intervals also kidney uptake ratios are demonstrated as the excretion route

criteria. It has been shown that after 24h post injection the increased highly-receptor expressing organ uptake is observed enough for imaging in half-life range of Indium-111.

Coinjection of 50  $\mu\text{g}$  AMD3100 per rats at 2 and 4 h post injection significantly reduced the possible uptake, thus demonstrating specificity of CXCR4-mediated  $^{111}\text{In}$ -AMD3100 as shown in Fig. (6). Bone uptake reduction from 6 to less than 2% can be a cause of bone marrow receptor blockade. Also the same behavior is observed in lung from 7 to 3% in 2h. Liver uptake reduced from 12 to less than 8% in 2 h.

**Table 1.** Various critical organ:blood uptake ratios for  $^{111}\text{In}$ -AMD3100 2-48 h post injection.

Ratios	2h	4h	24h	48h
kidney:blood	4.97	4.75	34.91	8.63
spleen:blood	0.71	0.47	5.11	2.56
lung:blood	1.25	0.82	2.08	1.79
liver:blood	1.85	1.57	10.63	5.72



**Fig. (6).** Percentage of injected dose per gram (% ID/g) of  $^{111}\text{In}$ -AMD3100 in rats tissues at 2-48 h post-injection based on 172 keV photopeak (n=3).

### Imaging of $^{111}\text{In}$ -AMD3100 in Rats

$^{111}\text{In}$ -AMD3100 imaging in the rats showed highly comparable results with tissue dissection studies, 48 h after injection high intestine, liver and spleen uptake were observed while in 24 h post injection kidney and bladder uptake was observed. Most of activity has been excreted through the kidneys according to reports [13] and the major remaining activity is associated to the presence of percentage of free indium possible produced by the slight degradation of the complex leading to free indium uptake in the liver at 24h Fig. (7).

### CURRENT & FUTURE DEVELOPEMENTS

Total labeling and formulation of  $^{111}\text{In}$ -AMD3100 took about 60 min (radiochemical purity >97% ITLC/HPLC, specific activity: 35-40 GBq/mmol) [10]. Radiolabeled complex showed good stability at room temperature and in human serum (37°C) for 24 h. Log P for the complex was -1.18 at the pH.7. The biodistribution of the labeled compound in vital organs of wild-type rats was determined [9] up to 48 h demonstrating kidneys as the major route of

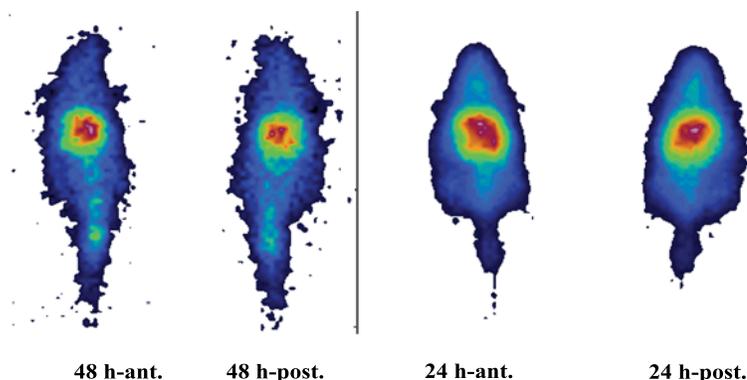


Fig. (7). Imaging of normal rats post injection of [ $^{111}\text{In}$ ]-AMD3100 (3.7-4 MBq, 100-110  $\mu\text{Ci}$ ).

excretion. Considering lungs, spleen and liver as the CXCR4 rich target organs the best target: non target ratios (spleen: blood, lung: blood and liver: blood) were obtained 24 h post injection (5.11, 2.08 and 10.63 respectively). [ $^{111}\text{In}$ ]-AMD3100 can be a good candidate for CXCR4 PET imaging for many cancers expressing these receptors.

#### CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

#### ACKNOWLEDGEMENTS

This study has been supported by Tehran University of Medical Sciences, Tehran, Iran; Grant no. 18308.

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