Indium-111 maltolate complex as a cell labeling agent for SPECT imaging

Original Article

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ABSTRACT

Introduction: Due to the cell labeling potentials of various metal-maltol complexes, the idea of developing a possible cell labeling imaging agent using single photon emission computed chromatography (SPECT) with [^{111}In]-maltolate complex has been investigated.

Methods: [^{111}In] labeled 3-hydroxy-2-methyl-4H-pyran-4-onate ([^{111}In]-maltolate) was prepared using freshly prepared [^{111}In]$InCl_3$ and 3-hydroxy-2-methyl-4H-pyran-4-onate in a sodium salt form in 25 min at 45°C. Stability of the complex was checked in final formulation and human serum for 48 h. The partition coefficient was calculated for the compound followed by biodistribution and imaging studies. [^{111}In]-maltolate was successively used in the radiolabeling of red blood cells for diagnostic studies.

Results: The complex was prepared successfully (radiochemical purity: >98% ITLC, >98% HPLC, specific activity: 15-17 GBq/mmol, log P=0.278). The complex is mostly washed out from the circulation through kidneys in 24h. the cell labeling efficacy was significant at the time of experiment.

Conclusion: [^{111}In]-maltolate can be an interesting cell imaging agent due to stability and cell avidity of metal maltol complexes, however, further biodistribution studies in various cells as well as diagnostic protocols is necessary.

Key words: Indium-111; maltolate; Cell labeling; Biodistribution

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INTRODUCTION

^{111}In or ^{99m}Tc labeled leukocyte scintigraphy has been shown to be useful in detection and diagnosis of infections and inflammatory lesions [1]. Results from multiple retrospective series indicate that leukocyte scintigraphy achieves sensitivity and specificity close to 90% in abdominal abscess detection [2]. ^{99m}Tc-HMPAO labeled leukocyte scintigraphy is now an accepted technique in the assessment of patients with inflammatory bowel disease [3].

Several ^{111}In-chelates are available for leukocyte labeling. Studies indicate that cells labeled with any of the chelates have sensitivity for infection of 90% to 95% when imaged at 24 hours postinjection [4]. However the search for the development of new cell labeling agent using SPECT [5,6] as well as positron emission computed chromatography (PET) [7,8] are still continuing.

Various 6-(alkoxymethyl)-3-hydroxy-4H-pyran-4-ones with potential for the chelation of indium(III) has been synthesized and evaluated for their ability to label human leucocytes with ^{111}In. The leucocyte labeling efficiencies of the selected ligands were greater and the in-vitro plasma stabilities were similar to that of ^{111}In-tropolonate. These results suggest that the new bidentate ligands may offer advantages over those currently used for cell-labeling, however the toxicity of the ligands in some cases were significant [9].

Maltol (3-hydroxy-2-methyl-4-pyrone), is a naturally occurring, non-toxic compound and common food additive. Maltol metal complexes stability arises from maltol deprotonation and its behaviour as an anionic, bidentate metal chelator at neutral to basic pH levels, forming strong bidentate/tridentate chelate with indium, gallium, iron, zinc, aluminum, vanadium [10] and lanthanides [11].

Some of maltolate metal complexes are reported as biologically active compounds including gallium-maltolate for lymphoma treatment [12] and aluminum-maltolate with apoptotic cell death [13]. Maltol and its derivatives have been used in treatment of iron overload disorders such as haemochromatosis and thalassemia major [14] and aluminium toxication [15]. Recently Sm-153 maltolate preparation and evaluation has been reported to demonstrate the biodistribution of final detoxified complex [16], and also Ga-67 maltolate complex as a possible tumor imaging agent [17].

Various maltol derivatives have been chelated to gallium (III), indium (III) and lanthanide (III) ions [18], however no biological properties were reported. Indium-111 maltol derivatives have been prepared previously for cell labeling showing advantages over oxine and tropolone as indium platelet labeling agents with less damage to platelets in the labeling process [19].

For RBS labeling, ^{99m}Tc is used more than 111In. However, RBC labeling in this work was performed as a cell model to demonstrate the possibility of accumulation of the complex in a human cell.

Due to metal maltol complexes stability reports [18] and the possible application of maltol as a safe, non-toxic and non-expensive complexing agent, the idea of developing a possible cell labeling imaging agent for SPECT (single photon emission computed tomography) by incorporating ^{111}In (half life:67 h, major photopeak at 172 keV) into a suitable anionic maltolate complex has been investigated (Fig. 1).

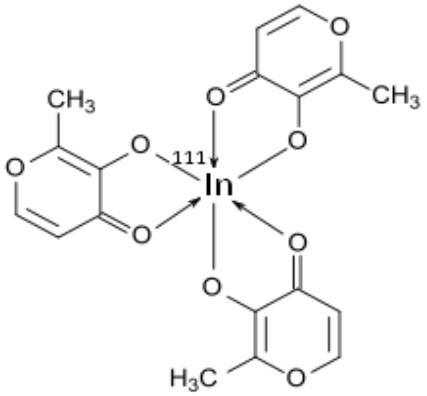

Fig 1. Structure of *tris*(maltolato) ^{111}In (III)

METHODS

Production of ^{111}In was performed at the Agricultural, Medical and Industrial Research School (AMIRS), 30 MeV cyclotron (Cyclone-30, IBA) using ^{nat}Cd(p,x)^{111}In. Natural cadmium sulfate with a purity of >99% was obtained from Merck Co. Germany. All chemicals were purchased from Sigma-Aldrich Chemical Co. U.K.. Radio-chromatography was performed by Whatman paper using a thin layer chromatography scanner, Bioscan AR2000, Paris, France. Analytical HPLC to determine the specific activity was performed by a Shimadzu LC-10AT, armed with two detector systems, flow scintillation analyzer (Packard-150 TR) and UV-visible (Shimadzu) using Whatman Partisphere C-18 column 250 × 4.6 mm (Whatman Co. NJ, USA). Calculations were based on the 172 keV peak for ^{111}In. All values were expressed as mean ± standard deviation (Mean± SD) and the data were compared using student T-test. Animal studies were performed in accordance with the United Kingdom Biological Council's Guidelines

on the Use of Living Animals in Scientific Investigations, second edition.

Production and quality control of ^{111}In-$InCl_3$ solution

Cadmium electroplating over a copper surface was performed according to the previously reported method [20]. ^{111}In-indium chloride was prepared by 22 MeV proton bombardment of the cadmium target at a 30 MeV cyclotron, with a current of 100 µA for 48 min (80 µAh). After dissolution of the irradiated target by concentrated HBr, the solution was passed through a cation exchange dowex 50×8 resin, pre-conditioned by 25 ml of concentrated HBr. The resin was then washed by HBr concentrated solution (50 ml). In order to remove the undesired impurities of Cd and Cu, the resin was totally washed with double-distilled H_2O. Indium-111 was eluted with 1 N HCl (25 ml) as $^{111}InCl_3$ for labeling use. Gamma spectroscopy of the final sample was carried out counting in an HPGe detector coupled to a Canberra™ multi-channel analyzer for 1000 seconds.

The presence of copper was checked using acidic dithizone solution using colorimetric assay.

For cadmium impurities in the final solution alkaline dimethylglyoxime in presence of NaK tartrate was used according to the reported procedures [21,22].

Preparation of [^{111}In]-maltolate

The acidic solution of [^{111}In] In Cl_3 (2 ml, 111 MBq) was transferred to a 3 ml-borosilicate vial and heated to dryness under a flow of N2 gas at 50-60°C. Fifty microlitres of sodium maltolate salt in absolute ethanol (5 mg/ml ≈409 nmoles) was added to the indium-containing vial followed by the addition of acetate buffer pH 5.5 (450 microliteres). The mixture refluxed at 45°C for 25 min. The active solution was checked for radiochemical purity by ITLC and HPLC. The final solution was then passed through a 0.22 µm filter and pH was adjusted to 5.5-7.

Quality control of [^{111}In]-maltolate

Radio thin layer chromatography: A 5 µl sample of the final fraction was spotted on a chromatography Whatman No. 2 paper, and developed in mobile phase mixture, 10% NH_4OAc and methanol 1:1.

High performance liquid chromatography: HPLC was performed with a flow rate of 1 ml/min, pressure: 130 kgF/cm^2 for 20 min. HPLC was performed on the final preparation using a mixture of water:acetonitrile 3:2 (v/v) as the eluent by means of reversed phase column Whatman Partisphere (C_{18} 4.6 × 250 mm).

Determination of Partition coefficient

Partition coefficient (log *P*) of [^{111}In]-maltolate was calculated followed by the determination of *P* (*P*= the ratio of specific activities of the organic and aqueous phases). A mixture of 1 ml of 1-octanol and 1 ml of isotonic acetate-buffered saline (pH.7) containing approximately 3.7 MBq of the radiolabeled indium complex at 37°C was vortexed 1 min and left 5 min. Following centrifugation at >1200*g* for 5 min, the octanol and aqueous phases were sampled and counted in an automatic well-type counter. A 500 µl sample of the octanol phase from this experiment was shaken again two to three 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.

Stability tests

A sample of [^{111}In]-maltolate (37 MBq) was kept at room temperature for 2 days while being checked by ITLC at time intervals in order to check stability in final product using above chromatography system. For serum stability studies, to 36.1 MBq (976 µCi) of [^{111}In]-maltolate was added 500µl of freshly collected human serum and the resulting mixture was incubated at 37°C for 24h, Aliquots (5-µl) were analyzed by ITLC.

Cell labeling

In a typical run, healthy male volunteer blood samples (3 ml) were collected in sterile anticoagulant added polymer tubes. The samples centrifuged at 3000 rpm for 5 minutes then the serum was discarded. The cell pellets were reconstituted in 1 ml of PBS followed by the addition of the [^{111}In]-maltolate final solution (3 MBq). The samples were kept at 4, 25 and 37°C bath up to 3 hours. One -ml samples were taken at various time intervals (30, 60, 120 and 240 min) and centrifuged at the 3000 rpm for 5 min. The cell pellets were carefully washed with PBS and the washing solution was discarded. The activity of the cell pellet and the supernatants were counted in a dose calibrator and the ratio of cell/supernatants were determined (n=5).

RESULTS AND DISCUSSION

Radiolabeling

Because of the engagement of three maltolate groups around ^{111}In core in complex structure, tris (maltolato) ^{111}In (III) complex has more lipophilic character. Chromatographic system was used for the detection of the radiolabeled compound from the free indium cation. Compared to ^{67}Ga-maltolate complex no meaningful difference was detected in the

radiochemistry and quality control [17]. Using 10% NH_4OAc and methanol (1:1) mixture, free indium remains at the origin of the paper as a single peak, while the radiolabeled compound migrates to higher R_f (0.52) (Fig. 2).

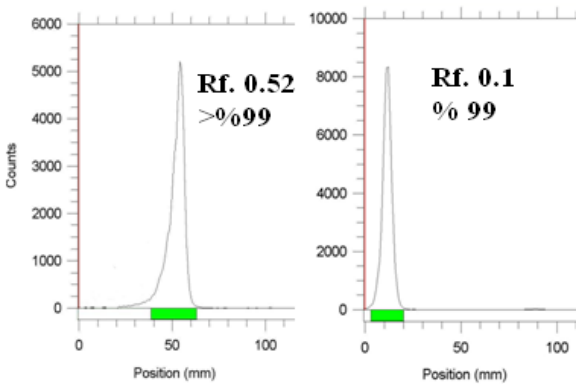

Fig 2. ITLC of [^{111}In]$InCl_3$ (right) and [^{111}In] maltolate (left) in a 10% NH_4OAc/methanol (1:1) mixture (left) as mobile phase on Whatman No.2 papers.

Although the ITLC studies approved the formation of radiolabeled compound, HPLC studies demonstrated the existence of radiolabeled species using both UV and scintillation detectors. The eluting compound at 6.9 min (scintillation detector) related to 6.5 min peak (UV detector) demonstrated a more lipophilic property of the complex (Fig. 3). Compared to ^{67}Ga-maltolate complex no meaningful difference was detected in the elution behavior, however the 111In complex is eluted faster than the 67Ga complex in the same elution system [17].

Partition coefficient of [^{111}In]-maltolate

As expected, the lipophilicity of the [^{111}In]-maltolate compound is rather medial. The measured octanol/water partition coefficient, *P*, for the complex was found to depend on the pH of the solution. At the pH.7 the log P was 0.278±0.04. Compared to ^{67}Ga-maltolate complex (log P. 0.4) a meaningful difference was detected [17]. Due to unknown reasons In-111 complex is more hydrophilic, this is also confirmed by fast elution in the HPLC column. According to the literature, there is no distinct range for specific cells and partition coefficient since all the cell membranes almost consist of the same macromolecules and substances. Table 1 demonstrates for partition coefficients for some well known radiolabeled ligands.

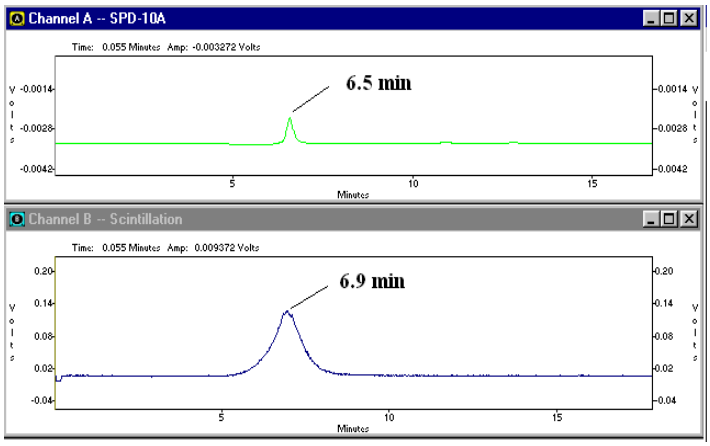

Fig 3. HPLC chromatograms of [^{111}In]-maltolate on a reversed phase column using acetonitrile:water (40:60) as eluent, up; UV chromatogram, down; scintillation chromatogram**.**

Table 1. The partition coefficients for some well known radiolabeled ligands.

radiolabeled complex	Log P	Application	reference
^{111}In-oxine	3.54	WBC	23
^{111}In-acetylacetone	7.93	WBC	23
^{111}In-tropolone	18.18	WBC	23
^{99m}Tc-HMPAO;	1.9	RBC	24
^{99m}Tc-ECD	1.64	RBC	23

Stability

The chemical stability of [^{111}In]-maltolate was high enough to perform further studies. Incubation of [^{111}In]-maltolate in freshly prepared human serum for 2 days at 37°C showed no loss of [^{111}In] from the complex. The radiochemical purity of complex remained at 98% for 2 days under physiologic conditions.

Cell labeling

Since it has been shown that the [^{111}In]-maltolate complex has lipophillic properties, it can easily pass through bi-layer phospholipids of cell membrane. In many cases the complexes stay intact in the intracellular space, resulting in easy leakage back to the outer cell space. Degradation of [^{111}In]-maltolate complex in the intracellular space is possible due to pH changes as well as reaction with cytoplasmic thiol-containing molecules. This can be an advantage of many radiometal labeled complexes as a cell labeling agent including [^{111}In]-maltolate, since the tracer is trapped in the cytoplasmic space. In any case of free or thiol-comlexed forms of cobalt the leakage to outer space has not been reported. Thus, [^{111}In]-maltolate can be used either in blood cell labeling if added directly to human blood cell fractions, or in the imaging of cerebrospinal flow for the detection of tumors and/or pathological abnormalities in CNS (if injected into subarachnoid space). Three various temperatures were chosen for the cell labeling. In our experiences, room temperature and 4°C were suitable since the labeling capacity (cell pellet count: supernatant count ratio) in the course of time was not linear and/or the data were not reproducible. At 37°C reproducible and linear data obtained with the course of time (Fig. 4). This might be explained by the fact that at this temperature natural existing enzymes or mechanism in cells are working properly so that the trapping of the tracer in cell occurs more efficiently. While at 25°C for instance, the leakage of the tracer 2 hours after labeling was observed (data not shown). The linearity of the plot also shows that the penetration of the tracer into the cells occurred by simple diffusion and no active mechanism was involved.

CONCLUSION

Total labeling time and formulation of [^{111}In]-maltolate was about 30 min (RCP >98% ITLC, >98% HPLC, specific activity: 15-17 GBq/mmol). The complex was stable in final formulation and also in human serum at least for 24 h. At the pH.7, the logP was 0.278.

Indium maltolate is stable in aqueous solutions between about pH 5 and 8, and it has significant solubility in water. Maltolate ligand is nontoxic, naturally occurring and easily synthesized. The properties of its complexes can be altered by changing the substituents on the ligand to optimize the biodistribution. In an effort to extend these properties to indium-111 radiopharmaceutical, radiolabeling and characterization of neutral complex of 2-methyl-3-oxy-4-pyronate (maltolate) with indium chloride form was presented. The linearity of the plot also shows that the tracer penetrates into the cells by simple diffusion mechanism and no active mechanism is involved. [^{111}In]-maltolate can be an interesting cell imaging agent due to stability and cell avidity of metal maltol complexes, however, further biodistribution studies in various cells as well as diagnostic protocols are necessary.

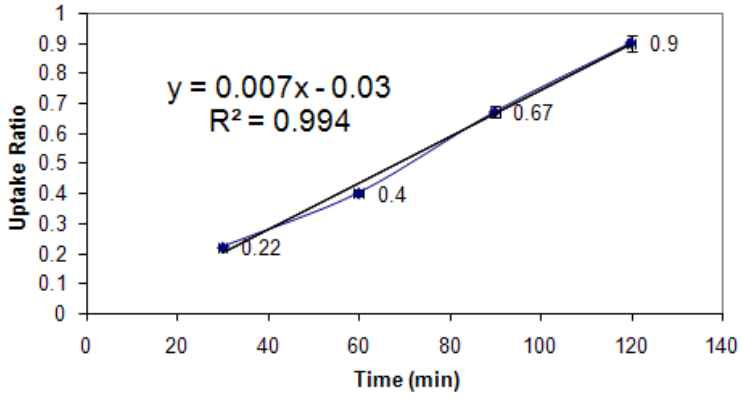

Fig 4. The rate of red blood cell labeling using [^{111}In]-maltolate at 37°C; n=5

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