Original Article

Radiosynthesis and biological evaluation of ^{111}In-tris [8-Hydroxy-2-methylquinoline] complex as a possible imaging agent

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ABSTRACT

Introduction: Due to the interesting pharmacological properties of radiolabeled metal oxine derivatives such as cell internalization, tumor avidity and antiproteosome activity, ^{111}In-*tris*[8-Hydroxy-2-methylquinoline] (^{111}In-HMQ) was developed in this study.

Methods: ^{111}In-HMQ was prepared using $^{111}InCl_3$ and 8-Hydroxy-2-methylquinoline (HMQ) for 60 min at 100°C (radiochemical purity: >99% ITLC, >99% HPLC, specific activity: 13-14 GBq/mmol). Stability of the complex was checked in final formulation and in the presence of human serum for 48 h. The partition coefficient was calculated for the compound (log P=0.68).

Results: The biodistribution of the labeled compound in vital organs of wild-type rats was studied using scarification studies and SPECT up to 24 h. A detailed comparative pharmacokinetic study for ^{111}In cation and ^{111}In-HMQ are performed up to 24h.

Conclusion: The complex is mostly cleaned from the circulation by kidneys and is a compound rapidly washing from the circulation. The biodistribution of the complex in tumor models is on-going.

Keywords: 8-Hydroxy-2-methylquinoline, In-111, Biodistribution, SPECT

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INTRODUCTION

Oxinate complexes are considered as antineoplastic compounds with various mechanisms. Some reports demonstrate the anti-proteasome and apoptosis inducing properties for these molecules (1). These interesting biological activities are not reported for free cations as well as oxine ligands by any research group independently.

Studies concerning the anti-proteasome activity demonstrated that metal-oxinate complexes form unknown complexes with proteasome and finally quinoline moiety of the complex inactivates the proteasome by an oxido-redox mechanism. Such a mechanism has been reported to induce apoptosis in a leukemic tumor models *in vivo* and *in vitro* (2).

On the other hand radiolabeled oxine complexes such as In-111 oxine has been widely used in the detection of various pathologies such as infections, inflammation (3) and thrombosis (4). Tumor-infiltrating lymphocytes with ^{111}In-oxine were infused to the cancer patients for tumor site detection (5).

We have already reported production and quality control of various oxinate radioisotope complexes for diagnostic purposes using Tl-201 (6), Ga-66 (7) as well as copper-61 which the latter demonstrated interesting tumor accumulation properties (8).

Most radiolabeled complexes of this group utilized oxine precursors, however some alkylated oxine compounds such as HMQ has demonstrated varied characters such as more lipophilicity (9) as well as versatile hepatic metabolism on the methyl group to produce carboxylate metabolites compared to oxine itself. On the other hand, the electron donor methyl group in the complex leads to more stability than normal oxine-metal complexes.

The interesting physical properties and availability of indium-111 make it an interesting nuclide for radiopharmaceutical research (10). Due to the interesting pharmacological properties of oxinates such as solubility in serum, rapid wash-out, tumor avidity and feasible complexation with various bi/tri-valent metals, the idea of developing a possible tumor imaging agent using SPECT (photon emission computed tomography) by incorporating ^{111}In into a suitable oxinate ligand, *i.e.* HMQ was investigated (Figure 1).

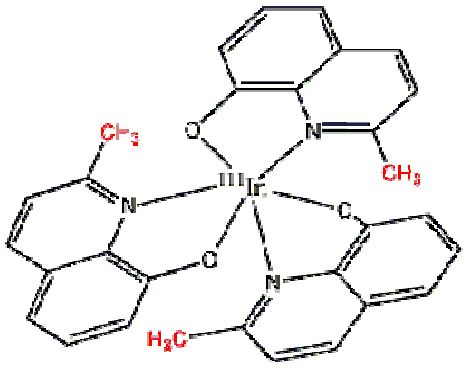

Figure 1. Possible structure of H_2HMQ (a) and ^{111}In-HMQ (b)

In this work we report, synthesis, radiolabeling, quality control, stability, partition coefficient determination and biodistribution studies (using SPECT and scarification) of ^{111}In-HMQ in wild-type rats. The time/activity diagrams for the labeled compound in vital organs have been plotted compared to indium cation.

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 reaction. 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.

Electroplating of the natural Cd targets

Cadmium electroplating over a copper surface was performed according to the previously reported method (11). Cadmium was electroplated over the copper backing according to the method given in the literature (12). Briefly, a mixture of $CdSO_4.8/3H_2O$, KCN, Brij and hydrazine hydrate with a final volume of 450 ml double-distilled water (DDH_2O) at pH=13 was used as the electroplating bath (constant current: 320 mA, stirring rate 780 rpm, time: 0.5 hours). After the deposition of an about 500 mg cadmium layer, the targets were wrapped in Parafilm® coatings to avoid atmospheric oxygen exposure. Finally, the target was sent for irradiation.

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

^{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 conc. HBr, the solution was passed through a cation exchange dowex 50×8 resin, pre-conditioned by 25 ml of conc. HBr. The resin was then washed by HBr conc. solution (50 ml). In order to remove the undesired impurities of Cd and Cu, the resin was totally washed with DDH_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 and cadmium impurities in the final solution was checked using acidic dithizone solution and alkaline dimethylglyoxime and NaK tartrate respectively according to the procedure (13, 14).

Quality control of the product

Control of Radionuclide purity: Gamma spectroscopy of the final sample was carried out counting in an HPGe detector coupled to a Canberra™ multi-channel analyzer for 1000 seconds.

Chemical purity control: This step was carried out to ensure that the amounts of zinc and copper ions resulting from the target material and backing in the final product are acceptable regarding internationally accepted limits. Chemical purity was checked by differential-pulsed anodic stripping polarography. The detection limit of our system was 0.1 ppm for both zinc and copper ions.

Preparation of ^{111}In-HMQ

The acidic solution (2 ml) of $^{111}InCl_3$ (111 MBq, 3 mCi) was transferred to a 3 ml-borosilicate vial and heated to dryness using a flow of N_2 gas at 50-60°C. Fifty microlitres of HMQ 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 100°C for 60 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-HMQ

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-HMQ 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

The stability of the complex was checked according to the conventional ITLC method (15). A sample of ^{111}In-HMQ (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-HMQ was added 500μl of freshly collected human serum and the resulting mixture was incubated at 37°C for 48 h, aliquots (5-μl) were analyzed by ITLC.

Biodistribution in wild-type rats

The distribution of the radiolabelled complex among tissues was determined for wild-type rats immediately after imaging. 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. The animals were deceased using the animal care protocols at selected times after injection (2 to 24h), the tissues (blood, heart, lung, brain, intestine, faeces, skin, stomach, kidneys, liver, muscle and bone) were weighed and rinsed with normal saline and their specific activities were determined with a HPGe detector equipped with a sample holder device as percent of injected dose per gram of tissues. Blood samples were rapidly taken from rodent aorta after scarification.

Imaging of wild-type rats

Images were taken 2, 4 and 24 hours after administration of the tracer 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×400 mm.

RESULTS AND DISCUSSION

Radiolabeling

Because of the engagement of NH and OH polar functional groups in its structure, labeling of HMQ with indium cation affects its chromatographic properties and the final complex is more lipophilic.

Chromatographic system was used for the detection of the radiolabeled compound from the free indium cation. 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 (0.65) (Figure 2).

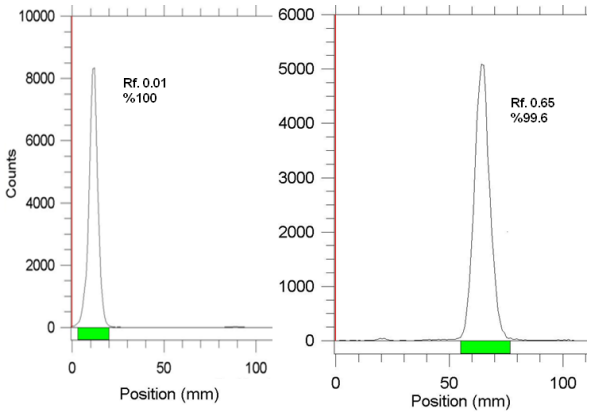

Figure 2. ITLC of $^{111}InCl_3$ (left) and ^{111}In-HMQ (right) in a 10% NH_4OAc and methanol 1:1 mixture (left) as mobile phase on Whatman No.2 papers.

ITLC studies approved the production of a single radiolabeled compound, HPLC studies also demonstrated the existence of only one radiolabeled species using both UV and scintillation detectors. A more fast-eluting compound at 7.72 min (scintillation detector) related to 7.84 min peak (UV detector) demonstrated a more lipophilic compound compared to In cation and unlabeled compound. Free In-111 cation eluted at 1.22 minutes (not shown) (Figure 3).

Partition coefficient of ^{111}In-HMQ

As expected from the chemical formula in Figure 1, the lipophilicity of the ^{111}In-HMQ compound is not rather high due to the ionic nature of the radiocomplex. 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 logP was 0.68. The water solubility of the radiocomplex leads to less unnecessary uptakes in tissues including liver and fat and faster kidney wash-out.

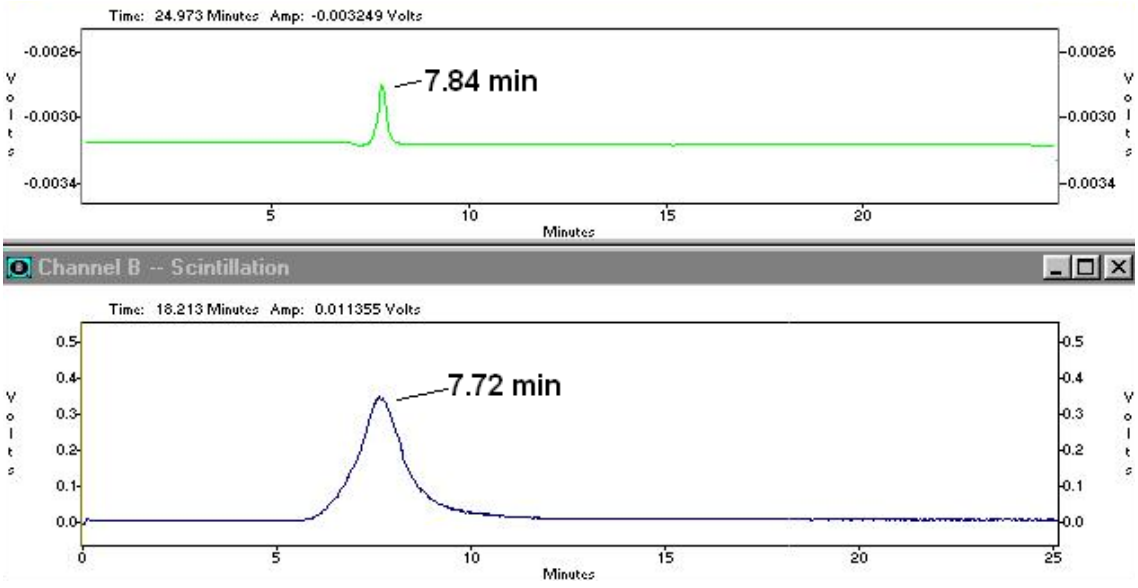

Figure 3. HPLC chromatograms of ^{111}In-TPFP on a reversed phase column using acetonitrile:water 40:60, up; UV chromatogram, down; scintillation chromatogram.

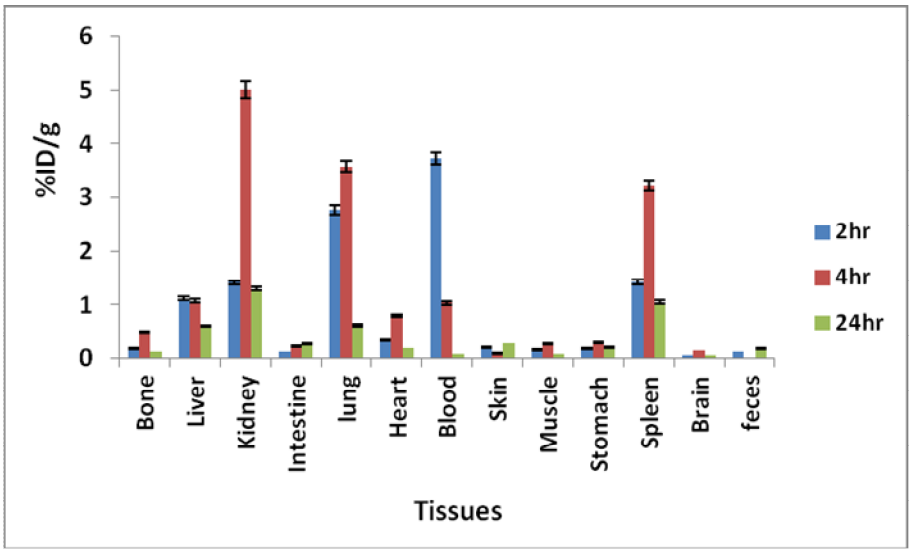

Figure 4. Biodistribution of ^{111}In-HMQ (1.85 MBq, 50μCi) in wild type rats 2,4 and 24 h after iv injection via tail vein (ID/g%: percentage of injected dose per gram of tissue calculated based on the area under curve of 172 keV peak in gamma spectrum) (n=3).

Stability

The chemical stability of ^{111}In-HMQ was high enough to perform further studies. Incubation of ^{111}In-HMQ in freshly prepared human serum for 24 h at 37°C showed no loss of ^{111}In from the complex. The radiochemical purity of complex remained at 98% for 24h under physiologic conditions.

Biodistribution in wild type rats

For better comparison biodistribution study was performed for free In^{3+}. As reported previously, ^{111}In is excreted majorly from gastrointestinal tract (GIT), thus colon and stool activity content are significant while blood stream activity is high at 2-4 h followed by reduction in 24. Bone uptake is also observed after 24 h post injection.

Indium cation almost mimics the ferric cation behavior and is rapidly removed from the circulation and is accumulated in the liver, also a major fraction is excreted through the urine as a water soluble cation. (data not shown).

The radiolabeled compound biodistribution is also demonstrated in Figure 4. Due to water solubility of the oxinate compound as an ionic complex, the major activity in 2 hours post injection is present in kidneys and spleen thus the major route of excretion for the labeled compound is urinary tract after 24h. Low intestinal activity demonstrates the low hepatobiliary excretion route.

The comparative study of vital organs uptake for ^{111}In-HMQ and $^{111}InCl_3$ and the kinetic pattern differences for both species. ^{111}In cation is accumulated in the liver in the first 24h post injection slightly, while ^{111}In-HMQ first major excretion route is through the liver.

Since the urinary tract is a major route of excretion of the oxinates, the kidney is the major excretory organ and shows high activity for the labeled compound esp. after 4h while in the case of In-111 the activity content is low and almost unchanged until 24h.

Due to rapid excretion of the complex from the blood, a rapid reduction of the blood activity content is observed in the first 4h, which in case of free cation the amount is low, however slowly increased.

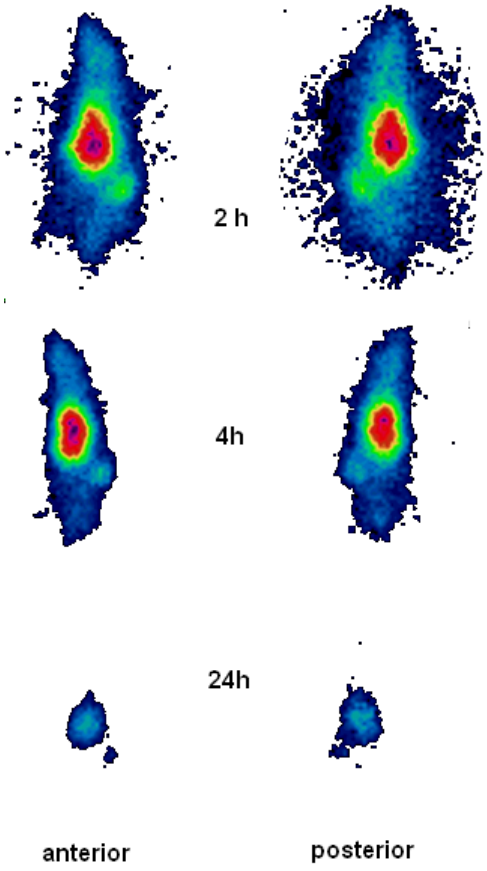

Figure 5. SPECT images of ^{111}In-HMQ (90 MBq, 22 µCi) in wild-type rats 2h (a), 4h (b) and 24h (c) post injection, (anterior scan; left and posterior scan; right).

Both compounds are excreted through the kidney, however in case of the labeled compound the excretion gets to a maximum in 4 h significantly, while In-111 cation is excreted slowly in 24 h, with an almost steady manner.

As a metal cation In-111 has significant spleen uptake while the labeled complex is accumulated in the spleen gradually up to 3% in 4h.

As a possible imaging agent the liver uptake of the complex is very low, that allows administration of higher doses with lowest possible radiation hazard to the subject as well as reduction in abdominal uptake leading to better diagnosis of abdominal lesions.

Imaging of wild-type rats

^{111}In-HMQ imaging in the wild-type rats showed a distinct accumulation of the radiotracer in the chest region all the time after injection. Most of the activity is washed out from the body after 24h and the picture contrast weakened (Figure 5). While a typical In-111 scan is usually high chest and abdomen activity accumulation remaining at least 48 hours in the rat body.

CONCLUSION

Total labeling and formulation of ^{111}In-HMQ took about 60 min (RCP >99% ITLC, >99% HPLC, specific activity: 13-14 GBq/mmol). The complex was stable in final formulation and in the presence of human serum at least for 48 h. At the pH.7, the log P was 0.68. The biodistribution of the labeled compound in vital organs of wild-type rats was studied using scarification studies and SPECT imaging up to 24 h.

A detailed comparative pharmacokinetic study performed for ^{111}In cation and ^{111}In-HMQ was studied for 24 h. The complex is mostly washed out from the circulation through kidneys. The SPECT images of the radiolabeled compound demonstrated high abdomen uptake 2h-24h post injection which is in agreement with biodistribution data. Higher water solubility of the complex due to ionic nature of the complex is an

advantage for rapid wash-out of the complex from the animal body leading to a enhanced target:non-target ratio. High biological stability of the complex compared to the other oxinates allows better accumulation time for imaging. The accumulation of the tracer in tumor models is currently under investigation.

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