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Evaluation of labelling conditions, quality control and biodistribution study of ^{99m}Tc-5-aminolevulinic acid (5-ALA): a potential liver imaging agent

Kalim Ullah Khan · Samina Roohi · Mohammad Rafi · Rizwana Zahoor · Zafar Iqbal · Mushtaq Ahmad

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Abstract Labelling of 5-aminolevulinic acid (5-ALA) with ^{99m}Tc was achieved by using $SnCl_2 \cdot 2H_2O$ as reducing agent. Radiochemical purity and labelling efficiency was determined by instant thin layer chromatography/paper chromatography. Efficiency of labelling was dependent on many parameters such as amount of ligand, reducing agent, pH, and time of incubation. ^{99m}Tc labelled 5-ALA remained stable for 24 h in human serum. Tissue biodistribution of ^{99m}Tc-5-ALA was evaluated in Sprague–Dawley rats. Biodistribution study (% ID/g) in rats revealed that ^{99m}Tc-5-ALA was accumulated significantly in liver, spleen, stomach and intestine after half hour, 4 and 24 h. Significant activity was noted in bladder and urine at 4 h. High liver uptake of ^{99m}Tc-5-ALA makes it a promising liver imaging agent.

Keywords ^{99m}Tc-ALA · Aminolevulinic acid · Quality control · Biodistribution · Liver imaging agent

Introduction

The availability of short lived technetium-99m (^{99m}Tc; half-life = 6 h) from the ^{99}Mo/^{99m}Tc generator, as the daughter product of long lived molybdenum-99 (^{99}Mo; half-life = 67 h), is a major factor behind the growth of diagnostic nuclear medicine. There are numerous ^{99m}Tc complexes useful for diagnostic procedures, of which over thirty agents are used in clinical studies. The search for new and more efficacious ^{99m}Tc radiopharmaceuticals has been a continuous process for the last 50 years.

5-Aminolevulinic acid (5-ALA) is prodrug biologically inactive compound which can be processed in the body to produce a drug enzymatically transformed into the protoporphyrin IX (PpIX) photosensitizer. In addition its cellular permeability, increase stability and limit side effect associated with the use of 5-ALA. 5-ALA was used for the treatment of Basel cell carcinoma in 1990, and it was administered topically [1]. At 630 nm wavelength PpIX has low absorption, so 5-ALA has been used for the treatment of skin basal carcinoma cells as well as gastrointestinal adenocarcinoma. It is also used for tumor diagnosis [2, 3]. 5-ALA is effective and gives promising results in dermatology for the treatment and diagnosis of neoplastic skin tissues [2].

The major limitation in the treatment of cancer by the photodynamic therapy method is quenching of fluorescence by body fluids, blood and normal tissues that restrains the data acquisition and recording of the fluorescence radiographically or photographically. Hence radiolabeled porphyrins were suggested to be better alternative for tumor detection [4]. These include radiolabeled with Cobalt-57 [5], Copper-64 [6, 7], Neodymium-140 [8], Gallium-67 [9] and Technetium-99 m [10, 11].

Considerations of cost, availability, and superior properties for imaging make ^{99m}Tc a better label than other radionuclides, hence labeling of 5-ALA was studied with ^{99m}Tc and biodistribution was performed in normal rats. These studies show that the labeled compound may be used for liver imaging.

K. U. Khan · M. Rafi
Pakistan Institute of Engineering and Applied Sciences, Islamabad, Pakistan

S. Roohi · R. Zahoor · Z. Iqbal · M. Ahmad (✉)
Isotope Production Division, Pakistan Institute of Nuclear Science and Technology, P.O. Nilore, Islamabad 45650, Pakistan
e-mail: amushtaq1@hotmail.com

Experimental

Materials and methods

5-ALA was obtained from Sigma-Aldrich, Germany. ^{99m}Tc was obtained from locally produced fission based PAKGEN ^{99}Mo/^{99m}Tc generator system. Rats (Sprague–Dawley, weight: 150–200 g) were obtained from National Institute of Health (NIH), Islamabad. The animal Ethics Committee of the institute gave approval for the animal experiments. All the chemicals used were AR grade and purchased from Merck, Germany.

Radiolabelling of 5-ALA with ^{99m}Tc

Stock solution of 5-ALA was prepared by dissolving 2 mg of 5-ALA in 1 mL of distilled water. To protect 5-ALA from direct light solution was covered with aluminium foil and carbon paper and stored in a refrigerator at 4 °C. From stock solution 100 μg of 5-ALA was pipette out in a clear vial with the help of micropipette. Known amount of Stannous chloride dihydrate was dissolved in 0.1 mL of concentrated HCl and diluted with distilled water to get required amount of reducing gent. To the ligand varying amount of $SnCl_2{\cdot}2H_2O$ and 1 mL of $^{99m}TcO_4^-$ was added. The pH of the solution was adjusted with the dilute NaOH solution. The mixture was then incubated for different time periods at room temperature (23 °C ± 2) for labelling purposes. At least five set of experiments were performed for each point. Means, standard deviations, percentages and student *t* tests were used for the calculation of statistical variations.

Optimization of labelling conditions

Efficiency of labelling of 5-ALA with ^{99m}Tc was studied by varying pH, amount of reducing agent, ligand and time of incubation. Labelling was performed at different pH (2–9) while amount of reducing agent was changed as (5, 10, 15, 20, and 30 μg) and amount of ligand was varied from 50, 75, 100, 150, and 200 μg. After labelling the stability of complex was checked up to 24 h. Labelled 5-ALA, free and hydrolyzed/reduced activity was checked by Whatman paper number 3 and instant thin layered chromatography (ITLC/SG).

Quality control

Labeling efficiency and radiochemical purity of ^{99m}Tc-ALA was assessed by instant thin layer chromatography (ITLC/SG) and ascending paper chromatography (Table 1). Free $^{99m}TcO_4^-$ in the preparation was assessed by using acetone as a mobile phase and Whatman paper (No. 3) as a stationary phase. Hydrolyzed or Reduced activity was determined by instant thin layer chromatography (ITLC/SG) strips as stationary phase and 0.05 M NaOH as a mobile phase. The stability of ^{99m}Tc-5-ALA was also checked up to 24 h at room temperature.

Table 1 Biodistribution (% ID/g tissue) of ^{99m}Tc-5-ALA in normal rats ($n = 3$)

Organ	0.5 h	4 h	24 h
Liver	43.94 ± 4.3	22.64 ± 2.32	0.89 ± 0.15
Spleen	6.13 ± 1.5	5.85 ± 1.64	0.05 ± 0.003
Stomach	0.64 ± 0.3	4.38 ± 1.28	0.83 ± 0.05
Intestine	0.32 ± 0.13	13.40 ± 2.26	0.94 ± 0.08
Lungs	2.82 ± 0.6	3.99 ± 0.54	0.13 ± 0.013
Kidney	1.42 ± 0.28	5.00 ± 1.2	1.80 ± 0.01
Femur	0.16 ± 0.05	0.49 ± 0.003	0.14 ± 0.06
Urine	0.11 ± 0.01	17.38 ± 3.2	0.03 ± 0.01
Bladder	1.20 ± 0.09	2.84 ± 0.5	3.05 ± 0.48
Heart	0.70 ± 0.06	0.85 ± 0.21	0.03 ± 0.007
Brain	0.033 ± 0.002	0.112 ± 0.005	0.09 ± 0.001
Blood	0.241 ± 0.006	0.221 ± 0.007	0.121 ± 0.005
Body	0.32 ± 0.01	21.74 ± 2.78	4.65 ± 0.18

Paper electrophoresis

Paper electrophoresis of ^{99m}Tc-5-ALA was performed using Whatman No. 1 paper as support and 0.05 M, Na-phosphate Buffer (pH 6.9) as a electrolyte. The sample was run at constant voltage of 300 V for 1 h. The paper strip was then scanned by 2π scanner. For comparison, a sample of $Na^{99m}TcO_4$ was also run under identical conditions. Migration of activity towards electrodes was checked.

Stability in human serum

Normal human serum 1 mL was mixed with 0.2 mL of ^{99m}Tc labelled 5-ALA. The mixture was incubated at 37 °C. Sample was taken during incubation after different time intervals upto 24 h and subjected to instant thin layer and paper chromatography. Any increase in the impurity was considered to be due to degradation of labeled compound.

Biodistribution in normal rats

Biodistribution study was performed in normal rats. The rats were placed in a covered jar containing cotton swab dipped in chloroform. After being anaesthetized, 0.2 mL of the ^{99m}Tc-5-ALA (~100 MBq) was injected in the tail veins of anesthetized rats. Rats were killed after 0.5, 4 and 24 h time intervals with an overdose of chloroform and

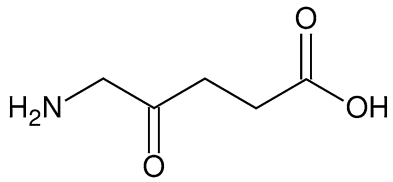

Fig. 1 5-aminolevulinic acid (5-ALA)

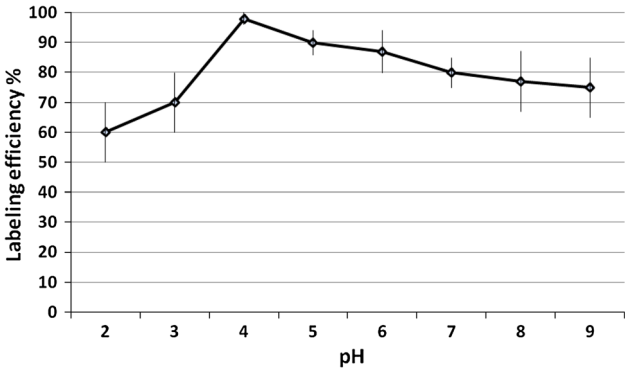

Fig. 2 Effect of pH on the labelling efficiency (%) of ^{99m}Tc-5-ALA ($n = 5$)

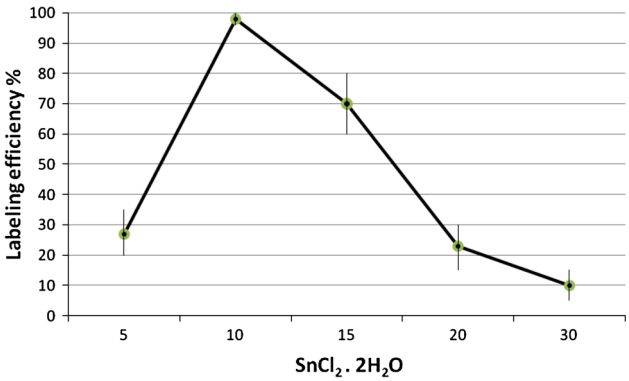

Fig. 3 Effect of Reducing agent on the labeling efficiency (%) of ^{99m}Tc-5-ALA ($n = 5$)

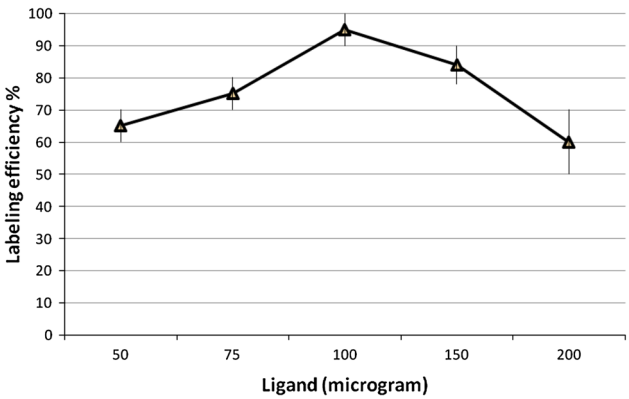

Fig. 4 Effect of ligand amount on the labeling efficiency (%) of ^{99m}Tc-5-ALA ($n = 5$)

then dissected. After dissection, the rats were weighed; 1 ml blood collected from the heart and a sample of urine was collected. Apart from that, different organs like liver, heart, spleen, stomach, lungs and kidney were removed. The distribution of activity in various organs was determined by well-type gamma counter (Ludlum® USA). The results were expressed as percentage of injected dose per organ (tissue) per gram (% ID/organ/g).

Results and discussion

In order to form complex with reduced ^{99m}Tc, the 5-ALA contains electron donors like nitrogen and oxygen to form bonds with metal (Fig. 1). Therefore it is assumed to be a chelate complex with one or more than one ALA ligands attached to reduced ^{99m}Tc. During preparation of ^{99m}Tc-5-ALA, the labelling efficiency, radiochemical purity and the stability were assessed by ascending paper chromatography and instant thin layer chromatography. In paper chromatography the mobile phase was acetone. In this system free ^{99m}TcO4-moved towards the solvent front (Rf = 1) while ^{99m}Tc-5ALA and reduced/hydrolyzed ^{99m}Tc remained at the origin. In another system, ITLC/SG was used as stationary phase, while the mobile phase was 0.05 M NaOH. In this system free $^{99m}TcO_4^-$ and ^{99m}Tc-5-ALA moved towards solvent front (Rf = 1) leaving behind reduced/hydrolyzed ^{99m}Tc at the origin. Labelling efficiency was checked at different pH values (Fig. 2) and it was observed that at pH 2, the labelling efficiency was 50–70 % and that reached to its maximum value 98 ± 2 % at pH 4. At pH 7 and 9 it was 75–85 % and 65–85 % respectively. Hence further experiments were carried out at pH 4. The effect of various concentrations of $SnCl_2{\cdot}2H_2O$ on labelling efficiency is shown in Fig. 3. At the concentration of l0 µg of $SnCl_2{\cdot}2H_2O$ labelling efficiency was 98 % that decreased with increasing or decreasing concentration of reducing agent. Effect of concentration of 5-ALA (ligand) on labelling efficiency (Fig. 4) shows that at concentration of 50 µg, the labelling efficiency was 60–70 % while efficiency of 98 % was achieved at 100 µg concentration of 5-ALA that again dropped to 50–70 % by increasing the concentration upto 200 µg.

Optimum conditions of labelling were 100 µg of 5-ALA, l0 µg of $SnCl_2{\cdot}2H_2O$, pH 4 and 30 min of incubation time at room temperature (23 ± 2 °C). Paper electrophoresis results demonstrated that ^{99m}Tc-5-ALA did not move toward any electrode and >90 % of the activity

remained at point of origin. Therefore it is assumed that the ^{99m}Tc-5-ALA is uncharged specie. Stability of ^{99m}Tc-5-ALA was assessed at room temperature. It was revealed that radiolabelled 5-ALA remained stable upto ~100 % till 24 h at room temperature and also in human serum. Loss of stability in the blood can result from a number of different causes such as thermodynamic instability of the complex followed by transchelation to other complexing proteins such as albumin or transferral and attack by enzymes such as peptidases [12].

The tissue distribution of ^{99m}Tc-5-ALA in normal rats/g tissue is given in Table 1. After intravenous injection, ^{99m}Tc-5-ALA was rapidly distributed. The results of the biodistribution study revealed high accumulation in liver, spleen, intestine and kidney and urine showing renal and hepatobiliary excretion of the compound. Distribution of activity was also noted in lungs and stomach. The highest uptake of ^{99m}Tc-5-ALA approximately 44 %/g was found in liver after 0.5 h of injection which decreased to 23 % after 4 h and reached to its minimum value of 0.38 %/g after 24 h. The unlabelled (cold) 5-ALA is preferentially taken up by the liver, kidney, endothelials and skin as well as by malignant gliomas and metabolised to fluorescent PpIX [13]. The biodistribution results suggest that labeled and cold 5-ALA have same distribution pattern and radiometal did not change its original behavior. In initial stages of injection of ^{99m}Tc-5-ALA most of the activity was localized in the liver, hence ^{99m}Tc-5-ALA can be used for liver scintigraphy.

Further studies are warranted to evaluate ^{99m}Tc-5-ALA potential in volunteers and comparison with different types of Tc-99m colloids/complexes which are already in use for patient studies.

Conclusion

In this study 5-ALA was labelled with ^{99m}Tc followed by evaluation of its quality control procedure, optimization of labelling condition and biodistribution in normal rats and it is concluded that it can be used as liver imaging agent.

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