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^{99m}Technetium Radiolabeling and Biodistribution Studies of Some Peptide-Based Ligands

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Abstract: In the present study, three peptide based ligands viz. diethylenetriaminepentaacetic acid (DTPA) or pentetic acid, 2,3-diaminopropionic acid (DAPA), iminodiacetic acid (IDA) were radiolabeled with technetium-99m (^{99m}Tc) by stannous chloride reduction method leading to the formation of ^{99m}Tc-ligand chelates. The radiochemical purification of these chelate compounds was achieved by quantitative thin layer chromatography (TLC) with two different solvent systems. Then the chelates were administered intravenously to Swiss albino mice under proper experimental conditions to assess the biodistribution pattern. The biodistribution studies in mice demonstrated that liver, kidney and intestine are the most distributed organs; whereas blood and urine are the most distributed body fluids of all the radiolabeled ligands.

Key words: Technetium-99m (^{99m}Tc) · Radiochemical · Radiolabeling · Biodistribution

INTRODUCTION

Nuclear medicine is a branch of medicine and medical imaging that uses the nuclear properties of matter in diagnosis and therapy. More specifically, nuclear medicine is a part of molecular imaging because it produces images that reflect biological processes that take place at the cellular and sub-cellular level. Nuclear medicine procedures use pharmaceuticals that have been labeled with radionuclides (radiopharmaceuticals). In diagnosis, radioactive substances are administered to patients and the radiation emitted is detected. The diagnostic tests involve the formation of an image using a gamma camera or positron emission tomography. Imaging may also be referred to as radionuclide imaging or nuclear scintigraphy. Other diagnostic tests use probes to acquire measurements from parts of the body, or counters for the measurement of samples taken from the patient In nuclear medicine, nearly 95% of the radiopharmaceuticals are used for diagnostic purposes while the rest are used for therapeutic purpose. Radiopharmaceuticals usually have no pharmacological effects because in most cases they are used in tracer quantities [1, 2].

Technetium-99m is an almost ideal diagnostic tool. It has a half-life of about six hours. After six hours, only half of it remains as technetium-99m. The rest has broken down into another element. After 24 hours, only one-sixteenth of the original isotope remains. It breaks down and disappears very quickly [2].

Amino acids are known to be the building block of proteins but they serve many other functions in cell which are essential for life. It has been established that amino acids also play an important role in the growth of the tumor cells. The involvement of amino acid in different physiological process is versatile. This encourages many scientists to radiolabel amino acid based chelating agent with ^{99m}Tc and to study their physicochemical and biological behavior. Nature has designed peptides to stimulate, inhibit or regulate many body functions. The development of peptide-based radiopharmaceuticals for imaging a variety of tumors, infection/inflammation and thrombus has seen a new era in nuclear medicine [3].

Recently, a number of ^{99m}Tc-labelled bioactive peptides and peptide analogues have proven to be useful diagnostic imaging agents. Due to their small size, these molecules exhibit favorable pharmacokinetic characteristics, such as rapid uptake by target tissue and rapid blood clearance, which potentially allows images to

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be acquired earlier following the administration of a ^{99m}Tc-labelled peptide radiopharmaceutical. Peptides are important regulators of growth and cellular functions not only in normal tissue but also in tumors. So they are becoming radioligands of increasing interest in nuclear oncology for targeted tumor diagnosis and therapy. So development of new peptide radiopharmaceuticals is becoming one of the most important areas in nuclear medicine research [3, 4].

Therefore, in the present study, we have aimed that small peptide-like compounds i.e. peptide based ligands viz. diethylenetriaminepentaacetic acid or (DTPA) or pentetic acid, 2,3-diaminopropionic acid (DAPA), iminodiacetic acid (IDA) radiolabeled with ^{99m}Tc could be an important area of radiolabeling and consequent biodistribution studies in rodents.

MATERIALS AND METHODS

Reagents and Chemicals: $^{99}MoO_4$ as $Na^{99}MoO_4$ was obtained from Bhabha Atomic Research Centre (BARC), Trombay, India. All the other reagents and chemicals were of highest purity grade obtained commercially. Doubled-distilled water from all-glass-still was employed throughout the studies.

Extraction of ^{99m}Tc: Sodium molybdate ($NaMoO_4$), containing $NaTcO_4$ was first dissolved in 5(M) NaOH containing traces of sodium chromate ($NaCrO_4$). Equal volume of methylethylketone ($C_2H_5COCH_3$) was added and it was properly stirred, followed by separating the two layers in separating funnel. The organic layer, containing free pertechnate was purified by basic Al_2O_3 column chromatography and the aqueous layer of 5(M) NaOH, containing MoO_4^- may be reused. The organic layer was then heated to dryness and $^{99m}TcO_4^-$ was again dissolved in N_2-purged double-distilled water [5].

Nitrogen-Purged Water: 50 ml of double-distilled water was boiled to expel out dissolved O_2, then N_2 gas was passed through the water in ice-cold condition.

Stannous Chloride Solution: 10 mg tin chloride dihydrate was taken in a 10ml volumetric flask and dissolved in a single drop (approx. 0.05 ml) of 6(N) HCl. Then the volume was made up to 10 ml with nitrogen-purged water.

Animals: Adult male Swiss albino mice of about 2 months of age weighing 20 ± 2 g were housed in polyacrylic cages (38×23×10 cm) with not more than four animals per cage and maintained under standard laboratory conditions (temperature 25 ± 2°C, relative humidity 48 %, with dark/light cycle 12/12 h). They were allowed free access to standard dry pellet diet and water *ad libitum*. The mice were acclimatized to laboratory conditions for 10 days before commencement of the experiment.

Radiolabeling of the Peptide Based Ligands with ^{99m}Tc [6]

Preparation of ^{99m}Tc-DTPA Chelate

Preparation of DTPA Solution: 5 mg DTPA powder was dissolved in 1 ml N_2 purged water (minimum volume). pH was adjusted to 4 with 0.1(N) NaOH solution.

Radiolabeling with ^{99m}Tc isotope: The ligand (DTPA) was labeled with ^{99m}Tc by adding ^{99m}Tc-pertechnate (1-5 mCi, 0.1 ml) to the DTPA solution followed by immediate addition of 50 il $SnCl_2$, $2H_2O$ solution. $SnCl_2$, $2H_2O$ solution reduces Tc (VII) to Tc (V) oxidation state in presence of excess ligand.

Preparation of ^{99m}Tc-IDA Chelate

Preparation of IDA Solution: 10 mg IDA powder was dissolved in 1ml N_2 purged water (minimum volume). pH was adjusted to 4 with 0.1 (N) NaOH solution.

Radiolabeling with ^{99m}Tc isotope: The ligand (IDA) was labeled with ^{99m}Tc by adding ^{99m}Tc-pertechnate (1-5 mCi, 0.1ml) to the IDA solution followed by immediate addition of 50 il $SnCl_2$, $2H_2O$ solution. $SnCl_2$, $2H_2O$ solution reduces Tc (VII) to Tc(V) oxidation state in presence of excess ligand.

Preparation of ^{99m}Tc-DAPA Chelate

Preparation of DAPA Solution: 20mg DAPA powder was dissolved in 1ml N_2 purged water (minimum volume). pH was adjusted to 8 with 1(N) NaOH solution.

Radiolabeling with ^{99m}Tc isotope: The ligand (DAPA) was labeled with ^{99m}Tc by adding ^{99m}Tc-pertechnate (1-5 mCi, 0.1 ml) to the DAPA solution followed by immediate addition of 50il $SnCl_2$, $2H_2O$ solution. $SnCl_2$, $2H_2O$ solution reduces Tc (VII) to Tc (V) oxidation state in presence of excess ligand.

Radiochemical Purification Studies of the ^{99m}Tc Chelates by TLC: To ensure that the ^{99m}Tc chelates were formed properly, thin layer chromatography (TLC) was performed with the chelate compounds. TLC of the each of the ^{99m}Tc chelates were performed on two silica gel F 254 strips (Sigma Aldrich, 2.5 × 10 cm) and developed with acetone (solvent system A) and acetonitrile: water (1:1) (solvent system B). Then quantitative chromatograms were performed by cutting the strip into 5 uniform pieces and counting them in well-type gamma counter (ECIL, India) [7].

Biodistribution Study of ^{99m}Tc Chelates in Mice: Male Swiss albino mice (18, *n* = 6) were hydrated by intraperitoneal administration of isotonic saline (0.9 %, 3 ml/kg body weight) for 30 minutes. After that the ^{99m}Tc chelate in total volume 20 il (fraction I) was injected intravenously into these hydrated animals (20 il/mouse) via the tail vein. Injection is facilitated by washing the animal's tail with warm water. The mice were then sacrificed by decapitation at 30 minutes post injection time. The desired organs were collected after dissection. The urinary bladder was clamped, dissected carefully and transferred into counting vials. Blood samples were obtained by puncture of heart and it was assumed that the blood weight was 7 % of the body mass. The samples were counted against 1ml suitable diluted aliquotes of the injected solution (the injected volume of the chelate was taken in a 25 ml volumetric flask and volume was made up to the mark) and results were expressed as percentage of injected dose per gm of organ. Appropriate corrections were made for background counts. The percentage of the dose in each tissue or organ was calculated by dividing the counts in each tissue or organ by the total injected counts and by multiplying it with 100 the percentage of injected dose per gm of tissue or organ was obtained [8, 9].

Formulae for calculation for percentage of injected dose per gram of tissue/organ are stated below.

- Mean Standard Count = [Standard Count(before) + Standard Count(after)] / 2
- Standard Dose (S.D.) = Mean Standard Count × 25 (dilution factor)
- Final Injected Dose (I.D.) = Standard Dose – Tail Count
- % of injected dose in tissue/organ = (Count observed/ I.D.) × 100
- % of injected dose per gram of tissue/organ = [(Count observed/ I.D.) × 100] / weight of the tissue/organ

RESULTS

The radiochemical purity of ^{99m}Tc-IDA chelates were analyzed by TLC with two different solvent systems and counted by gamma-counter. The results are presented in Tables 1-3. The free pertechnetate moves with acetone

Table 1: Radiochemical purification studies of the ^{99m}Tc-DTPA chelate by TLC with two different solvent systems

Fractions	Acetone (cpm)	Acetonitrile/Water (cpm)
I	4566	488
II	3496	245
III	4506	254
IV	420	477
V	15	7010

Unit of measurement: counts per minute (cpm).

Table 2: Radiochemical purification study of the ^{99m}Tc-IDA chelate by TLC with two different solvent systems

Fractions	Acetone (cpm)	Acetonitrile / Water (cpm)
I	38598	3580
II	75	511
III	36	40
IV	16	1095
V	7	34502

Unit of measurement: counts per minute (cpm).

Table 3: Radiochemical purification study of the ^{99m}Tc-DAPA chelate by TLC with two different solvent systems

Fractions	Acetone (cpm)	Acetonitrile/Water(cpm)
I	25	6
II	1417	10
III	19	51
IV	10	134
V	15	1639

Unit of measurement: counts per minute (cpm).

Table 4: Biodistribution study of ^{99m}Tc-DTPA chelate in mice

Tissue/ Organ	Weight of tissue/ organ (g)	Count (cpm)	% dose of total tissue/ organ	% dose per gram tissue/ organ
Heart	0.14	1068	0.087	0.623
Blood	0.11	1735	2.29	1.30
Liver	1.27	12699	1.074	0.846
Lung	0.14	1360	0.112	0.8
Spleen	0.14	1151	0.09422	0.673
Muscle	0.07	1027	0.08372	1.196
Kidney	0.32	18232	1.544	4.824
Intestine	3.02	20731	1.756	0.581
Stomach	0.29	2259	0.188	0.65
Urine	-	544481	46.2	46.2

Biodistribution time = 30 mins.
Hydration time = 1 hr.
Dilution: 10µl→25ml→1ml.
Standard count (before) = 50717 (cpm) Standard dose = 1228962 (cpm)
Tail count = 49355 (cpm)
Standard count (after) = 50435 (cpm) Injected dose = 1178527 (cpm)
Background count = 40 (cpm)

Table 5: Biodistribution study of ^{99m}Tc-IDA chelate in mice.

Tissue/ Organ	Weight of tissue/ organ (gm)	Count (cpm)	% dose of total tissue/ organ	% dose per gram tissue/ organ
Heart	0.146	60	0.0651	0.446
Blood	0.065	88	2.559	1.462
Liver	1.91	1464	1.589	0.832
Lung	0.164	88	0.095448	0.582
Spleen	0.276	113	0.122544	0.444
Muscle	0.041	25	0.025502	0.622
Kidney	0.455	1094	1.187	2.61
Intestine	3.5	1791	1.943	0.555
Stomach	0.359	216	0.234	0.653
Urine	-	53166	57.704	57.704

Dilution: 30µl→25ml→1ml.
Standard count (before) = 4790 (cpm) Standard dose = 117875 (cpm)
Tail count = 25740 (cpm)
Standard count (after) = 4640 (cpm) Injected dose = 92135 (cpm)
Background count = 7 (cpm)

Table 6: Biodistribution study of ^{99m}Tc-DATA chelate in mice

Tissue/ Organ	Weight of tissue/ organ (gm)	Count (cpm)	% dose of total tissue/ organ	% dose per gram tissue/ organ
Heart	0.139	108	0.107	0.769
Blood	0.022	36	2.718	1.618
Liver	1.126	1499	1.484	1.318
Lung	1.126	90	0.088954	0.079
Spleen	0.236	60	0.059472	0.252
Muscle	0.037	156	0.154438	4.174
Kidney	0.354	640	0.634	1.79
Intestine	3.8	1565	1.55	0.469
Stomach	0.501	258	0.255	0.509
Urine	-	28669	28.38	28.38

Dilution: 30µl→25ml→1ml
Standard count (before) = 4640 (cpm) Standard dose = 114525 (cpm)
Tail count = 13508 cpm
Standard count (after) = 4522 (cpm) Injected dose = 101017 (cpm)
Background count = 25 (cpm)

whereas the ^{99m}Tc- complex remains at the positions where it has been applied. But both the free pertechnetate and the complex moves with the solvent in acetonitrile: water chamber. So from the present results of radiolabeling of all the three chelates with ^{99m}Tc, it can be concluded that in each case the percentage of the free pertechnetate is minute in comparison to the ^{99m}Tc-complex and the formation of the complex is almost perfect. The results of biodistribution of all ^{99m}Tc radiolabeled ligands in different organs and body fluids of Swiss mice are presented in Tables 4-6.

DISCUSSION

Nuclear medicine has evolved in recent time as a powerful tool in both medical diagnosis and therapy. The application of radiopharmaceuticals along with gamma camera or positron emission tomography is being extensively used today to diagnose severe pathological conditions like problems associated with peripheral and central blood flow, organ failure and cancer. The radiopharmaceuticals mostly used are peptide or peptide analog labeled with nuclides such as ^{99m}Tc, ^{67}Ga, ^{18}F, ^{123}I etc [10].

Technetium (atomic number: 43, atomic mass: 97.9072, family: Group 7 (VIIB), Transition metal) did not arrive on earth in any appreciable amount until 1937 when molybdenum atom was bombarded with deuterons by the scientists Carlo Perrier and Emile Serge. They eventually gave the name Technetium to the element, from the Greek word *technetos*, meaning 'artificial'. Technetium was the first element not found in the earth to be made artificially. It can now be made in much larger quantities, of at least a kilogram (two pounds) at a time [11].

Technetium-99m (^{99m}Tc) is a metastable nuclear isomer of technetium-99, symbolized as ^{99m}Tc. The "m" indicates that it is metastable nuclear isomer, i.e. it does not change into another element (transmutate) upon its "decay". ^{99m}Tc has a short half-life (6 h) and it quickly decays to its ground state of ^{99}Tc, which is relatively far-less radioactive compared to ^{99m}Tc. ^{99}Tc results in relatively less total radiation dose to the patient. When injected into

the body, it deposits in certain organs, such as the brain, liver, spleen and kidney. It also deposits in the bones. Technetium-99m gives off radiation that can be detected very easily. The amount and location of the radiation indicates problems with an organ or bones. Tehnetium-99m sends out clear, easily observed signals for a short time. Then, it is eliminated from the body [10, 11].

The radiation from external ^{99}Tc, however, is not harmful unless in close proximity or really internally present. The key issue is then that it may not be a problem now but its accumulation in the environment can do unexpectedly disastrous things. Internally ^{99}Tc is the only threat and ^{99}Tc is easily taken up into plants and animals but does not seem to do any damage as it is just as easily metabolized by biological chelating agents [3].

Pertechnate is the only compound of Technetium in oxidation state VII which is stable in aqueous solution. However in the presence of appropriate reducing agent $^{99m}Tc0_4$ is reduced to lower oxidation state-^{99m}Tc (probably III, IV, or V in most cases). In this lower oxidation state ^{99m}Tc forms stable complexes with a vast group of ligands. There are several agents available for the reduction of pertechnate. Among these agents are ascorbate-Fe (III), Fe (II), Sn (II) and electrophoresis etc. In this study, $^{99m}Tc0_4^-$ have been reduced to (V) oxidation state by $SnCl_2$, $2H_2O$ solution in presence of excess ligand (DTPA) so that as soon as ^{99m}Tc get reduced to (V) oxidation state it forms chelate with ligand. In most of the formulation ^{99m}Tc is present in 10^{-9}(M) concentration or less. During the reduction step some $^{99m}Tc0_4$ may be reduced to ^{99m}Tc (IV) state which ultimately results in formation of SnTc colloid by adsorption of Sn over TcO_2. Reportedly there is a chance of presence of unreacted free $^{99m}Tc0_4^-$ [11, 12].

Soon before the injection of the chelates into the animals, purity of the chelate must be confirmed. Purity is one of the most important parameter for radiopharmaceuticals. Purity check must be performed to ensure that only the isotope of interest is present and neither the parent nor daughter compounds nor other isotopic impurities are in any significant concentration. In the present study, radiochemical purification of ^{99m}Tc-ligand chelates was achieved by quantitative TLC with two different solvent systems and demonstrated excellent formation of chelate complexes indicating successful radiolabeling. The biodistribution studies of all these chelates (fraction I) in Swiss albino mice revealed that liver, kidney and intestine are the most distributed organs; whereas blood and urine are the most distributed body fluids of all the radiolabeled peptides. The outcome of the present study, although not reported elsewhere, is in concert with the previous investigations especially in biodistribution behaviour of ^{99m}Tc [13-15]. Further investigations involving sub-chronic/chronic toxicity and mutagenesis/carcinogenesis studies are necessary in this regard in pursuit of newer safe and effective peptide radiopharmaceuticals.

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