Macro-aggregates (MAA) of albumin for lung imaging. Studies on better tissue to background ratio, on MAA stability and reuse after its first preparation

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Abstract

The present study *was designed* to develop stable and economically competitive radioactive technetium-99m macro-aggregates of albumin (^{99m}Tc-MAA) which could be used for imaging of lungs. Macro-aggregates were freshly prepared and labeled with ^{99m}Tc pertechnetate by following the standard protocol which included incubation of formulation at 80° C for 10min. We studied 7 rats in every experiment. The rats were injected intravenously with ^{99m}Tc MAA and were sacrificed after 10min to study its distribution in the lungs and other non target tissues using gamma ray spectrometer. This standard protocol was further experimented upon in order to achieve high target to non target ratio. Different formulations were prepared by incubating them at 80 degrees for different incubation times of 5, 10, 15, 20, 25 and 30min. Formulation of MAA prepared by incubating at 80 degrees for 20min labeled with ^{99m}Tc showed the highest target to non target ratio. Another group of rats that received the above formulation were sacrificed after two additional time intervals of 5 and 15min. *The target to non target ratio* was high in animals sacrificed after 5min of injecting them with ^{99m}Tc the MAA formulation prepared by heating at 80 degrees for 20min as compared to animals sacrificed after 10 and 15min. Formulations of MAA following storage at room temperatures which varied from 5°C to 18°C, for different time durations 1, 2 and 9 days were also evaluated for their ability to be reused after reheating and labeling with ^{99m}Tc. The formulation of MAA kept for 9 days showed the best target to non-target ratio. *The present study suggests* that MAA once prepared can be reused following labeling with ^{99m}Tc even after 9 days of storage with better target to non target ratio as compared to storage timer period of 1 and 2 days.

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Introduction

Macro aggregates of albumin (MAA) labeled with ^{99m}Tc are being used worldwide as a radiopharmaceutical for the diagnosis of lung diseases and radionuclide venography [1]. The addition of ^{99m}Tc -pertechnetate ($^{99m}TcO_4^-$) obtained from ^{99}Mo-^{99m}Tc radionuclide generator enables the preparation of ^{99m}Tc-MAA. The pharmaceutical supplied by a manufacturing company, contains a lyophilized preparation of human MAA, stannous chloride and other substances as preservatives or stabilizers.

The widespread application of ^{99m}Tc-MAA in everyday diagnostic practice in nuclear medicine necessitates the development of simple, rapid, and possibly reusable MAA. Aggregation conditions of MAA may also be studied in varying temperatures and in varying incubation time periods.

The present study was planned to develop an in-house standardized protocol for the formation of stable, ^{99m}Tc-MAA, achieving higher target to non target ratio. Another aim of the present study was to check the reusability of the uniformly prepared ^{99m}Tc-MAA by storing the formulations of MAA for different time intervals and then testing them for in-vivo performance, following labeling with ^{99m}Tc. The standard procedures have been used for achieving better target to non- target ratio, and quality control. The present study may be useful to nuclear medicine clinical practice.

Materials and methods

Chemicals

All chemicals were purchased from Loba Chemie, Germany and Sigma Chemicals, USA and ^{99}Mo generator for the production of $^{99m}TcO_4^-$ was procured from the Board of Radiation and Isotope Technology- BRIT, Bhaba Atomic Research Centre Trombay, Mumbai, India.

Animals

Male wistar rats weighing 110±120g were procured from the central animal house, Punjab University, Chandigarh. The animals were housed in polypropylene cages in the departmental animal house under hygienic condi-

tions. All procedures were done in accordance with ethical guidelines for care and use of laboratory animals and were approved by our University Ethical Committee.

Preparation of MAA

Bovine serum albumin, 50 mg was added to 5mL of 10% sodium acetate solution which was further diluted to make a final volume of 21mL. To the above solution, 1mL of 0.5% $SnCl_2.2H_2O$ was further added and the final volume was made up to 25mL by adding distilled water which had pH of 5.5. The above solution was heated at 80°C on a water bath with constant stirring for 10min so as to complete the preparation of standard formulation. The above standard protocol was used for different sets of experiments in terms of preparation of MAA formulations at different incubation times. Various sets of preparations were made by constant stirring at 80°C but changing incubation times viz. 5, 10, 15, 20, 25 and 30min and by keeping the other conditions constant.

Storage and reusability of MAA formulations

The MAA formulations were prepared at a fixed incubation time of 20min at 80° C with constant stirring and were stored at room temperatures varying from 5-18°C for 1, 2 and 9 days. Furthermore, the MAA formulations stored at different time durations of 1, 2 and 9 days were reheated at 80° for 10min labeled with $^{99m}TcO_4^-$ and injected intravenously (i.v.) to the rats through the penile vein.

Radio labeling of MAA

A volume of 0.5mL of MAA from different formulations was mixed with equal volume of normal saline containing 55.5MBq of $^{99m}TcO_4^-$ radioactivity and the final preparation was then kept at room temperature for 10min in order to complete the labeling process.

Radiochemical punity

For the measurement of labeling efficiency of ^{99m}Tc with MAA, ascending paper chromatographic technique was applied. Very small aliquots of radiopharmaceutical preparations were spotted on Whatman's paper no.1 strips that were then placed in the chromatographic chamber using normal saline as a solvent, till the solvent front reached sufficient distance. Strips were then air dried and was cut into small pieces of 5mm size for the purpose of recording radioactivity. Values of Rf of free ^{99m}Tc and of the labeled form were determined.

Particle size measurements

Twenty micro-liters aliquots of MAA formulations were placed on a Neubauer haematocytometer and the size was determined by using a light microscope at 40X magnification.

Photo-peak settings and calibration of the gamma ray spectrometer

The gamma ray spectrometer supplied by Nucloeonix Pvt. Ltd. (Hyderabad, India) was used for counting the radioactivity. It was calibrated for the optimum peak setting of ^{99m}Tc. The high voltage of the counter was switched on, and set at the required voltage. The gain was appropriately adjusted and the photopeak for the standard ^{99m}Tc source was found out by increasing the baseline in small intervals until maximum counts were obtained. The windows (difference of upper and lower level discriminators) were then set and accordingly the baseline voltage was adjusted.

In vivo distribution of ^{99m}Tc-MAA

Male wistar rats were anaesthetized with light ether anesthesia and received 55.5MBq of ^{99m}Tc-MAA in a 1mL volume intravenously, through the penile vein. The same radioactivity was kept in a separate test tube to be used as a standard for the determination of % uptake values. Animals were dissected after 10min after the administration of ^{99m}Tc-MAA and the counts were recorded in the pre-calibrated gamma ray counter. The two other sets of experiments were also performed where animals were dissected 5 and 15min after the administration of ^{99m}Tc-MAA in order to achieve a better target to non target ratio for ^{99m}Tc-MAA. The specific activities were then calculated using the following formula:

Specific activity in an organ %=
Average observed counts in the organ- average background-(bg) counts X100 average counts in standard radioactivity-average bg counts X wt. of organ in mg
Specific activity in blood %=
Average observed counts in the blood fraction- average background-(bg) counts X100 average counts in standard radioactivity-average bg counts X volume of blood in ml

Results

The results obtained from various experiments are depicted in Tables 1-7. Table 1 shows radiochemical purity of ^{99m}Tc labeled MAA by ascending chromatography as well as particle size by haematocytometer. Tables 2 and 3 show biodistribution and target to non target ratio of ^{99m}Tc MAA in 7 rats, at different time periods of incubation. Tables 4 and 5 show biodistribution and target to non target ratio of ^{99m}Tc MAA in 7 rats, as a function of time of dissection Tables 6 and 7 show biodistribution and target to non target ratio of ^{99m}Tc MAA in 7 rats, following storage at different time intervals.

Table 1. *Radiochemical-purity analysis of ^{99m}Tc labeled MAA by ascending paper chromatography*

Rf (^{99m}Tc-MAA)	Rf ($^{99m}TcO_4^-$)	Binding percentage	Particle size in micrometers
0	1.0	96.2 %	5-100

Discussion

Lung perfusion imaging is a non-invasive method to visualize pulmonary vasculature. The underlying principle of perfusion imaging is pulmonary blood flow dependent distribution of particulate radiopharmaceuticals. Macro aggregates of human albumin labeled with ^{99m}Tc are used as one of the main clinical procedures in nuclear medicine for lung perfusion studies [3-6]. The status of ^{99m}Tc-MAA as the lung imaging radiopharmaceutical of choice is due to its particle size characteristics and its biologically metabolized nature.

Table 2. *Bio-distribution of ^{99m}Tc labeled MAA prepared at different time periods of incubation (time of dissection 10 min), (No. of rats =7)*

Organ	% Specific Activity 5min	% Specific Activity 10min	% Specific Activity 15min	% Specific Activity 20min	% Specific Activity 25min	% Specific Activity 30min
Liver	0.075	0.021	0.012	0.005	0.007	0.021
Lung	0.012	0.011	0.015	0.063	0.060	0.067
Blood	0.004	Bg	Bg	0.004	0.008	0.009
Kidney	0.008	0.015	0.015	0.044	0.017	0.019
Spleen	0.003	0.006	0.004	0.002	0.002	0.003

Table 3. *Target to non target ratio of ^{99m}Tc labeled MAA prepared at different time periods of incubation (time of dissection 10min), (No. of rats =7)*

Target (Lung) With respect to Non-target:	Target / Non target ratio after 5min	Target / Non target ratio after 10min	Target / Non target ratio after 15min	Target / Non target ratio after 20min	Target / Non target ratio after 25min	Target / Non target ratio after 30min
Liver	0.16	0.52	1.25	12.60	8.57	3.20
Blood	3.00	-	-	15.75	7.5	7.40
Kidney	1.5	0.73	1.00	1.43	3.5	3.50
Spleen	4.00	1.80	3.75	31.50	30.0	22.30

The MAA particle size is a key requisite attribute for the efficient performance and better target to non-target ratio. The range of size of the MAA particles recommended for efficient diagnostic applications varies from 10μm to 100μm, and needs to be checked prior to their administration to humans. Furthermore, the variation among the size of the particles of MAA to be injected should be kept as low as possible towards the 10μm [2]. These particles are cleared rapidly from the lungs by enzyme metabolism and mechanical break down due to haemodynamic pressure [7]. Moreover, the commercial availability of ready-to-use 'instant' ^{99m}Tc-MAA kit together with the availability of ^{99m}Tc from ^{99}Mo, $^{99m}TcO_4^-$ generator system, has enabled most nuclear medical centers to use ^{99m}Tc-MAA for lung scanning. However, use of these commercially available kits seems not to be cost-effective especially for nuclear medicine laboratories with poor economical background. Over the years, various techniques for the cost effective preparation of ^{99m}Tc-MAA have been reported in the literature [8-11].

The radiochemical purity of MAA formulation needs to be checked before the i.v. administration to humans or experimental animals. Several radiochemical impurities can exist in ^{99m}Tc-MAA preparations, such as reduced and hydrolyzed fraction of the ^{99m}Tc-Sn colloid, free pertechnetate ($^{99m}TcO_4^-$) and soluble ^{99m}Tc-albumin [12-14]. Each of these components is characterized by having its own specific *R*f value in a particular solvent. The radiochromatographic results are presented in (Table 1) showing that most of the ^{99m}Tc activity of labeled MAA remained at the starting point on the chromatographic paper. Further, the binding percentage of ^{99m}Tc MAA was 96.2% and the particle size of MAA ranged from 5 to 100 micrometers, which corroborated the labeling efficiency and the efficacy of the used standardized protocol.

Table 4. *Bio-distribution of ^{99m}Tc labeled MAA formulation prepared at 20min incubation time as a function of dissection time (No. of rats =7)*

Organ	% Specific activity 5min	% Specific activity 10min	% Specific activity 15min
Liver	0.019	0.022	0.016
Lung	0.051	0.027	0.026
Blood	Bg	Bg	0.000
Kidney	0.002	0.004	0.003
Spleen	0.002	0.001	0.001

Table 5. *Target to non target ratio of ^{99m}Tc labeled MAA formulation prepared at 20min incubation time as a function of dissection time (No. of rats =7)*

Target (Lung) With respect to Non-target	Target / Non target ratio after 5min	Target / Non target ratio after 10min	Target / Non target ratio after 15min
Liver	2.70	1.20	1.60
Blood	-	-	0.02
Kidney	25.50	6.70	8.70
Spleen	25.50	27.00	26.00

The particle size plays a key role in the efficient performance of MAA following i.v. injection. Various pulmonary shunt (right-to-left) studies also require a preparation in which more than 90% of the labeled MAA fragments should be within10 to 100μm range. The principle for using MAA is that it blocks the capillary bed in the lungs where particle size is the key parameter for the efficient in vivo result. The present study indicated indirectly that heating causes denaturation of MAA which further results in aggregation as the high temperature and time of heating regulate the particle size of albumin aggregates.

The standardized lung imaging protocol using ^{99m}Tc-MAA usually takes 10min after its i.v. administration to the subject before scanning of lungs is started. Interestingly, the best bio-distribution and target to non target ratio of ^{99m}Tc-MAA (Tables 4 and 5) was achieved 5min after the i.v. administration of ^{99m}Tc-MAA. The first aim of the study was to add to the existing knowledge that the formulation of macro-aggregates prepared following 20min of incubation at 80°C provides the best target to non target ratio after 5min of i.v. administration.

Table 6. *Biodistribution of ^{99m}Tc labeled MAA formulations following storage atdifferent time intervals (No. of rats =7)*

Organ	% Specific activity after 1 day of storage	% Specific activity after 2 days of storage	% Specific activity after 9 days of storage
Liver	0.018	0.034	0.002
Lung	0.069	0.071	0.069
Blood	0.010	0.015	0.010
Kidney	0.015	0.022	0.026
Spleen	0.009	0.006	0.002

The other aim of the present study was to check the

Table 7. *Target to non target ratio of ^{99m}Tc labeled MAA following storage at different time intervals (No. of rats =7)*

Target (Lung) with respect to Non-target	Target / Non target ratio after 1 day of storage	Target / Non target ratio after 2 days of storage	Target / Non target ratio after 9 days of storage
Liver	3.83	2.10	34.50
Blood	6.90	4.70	6.90
Kidney	4.60	3.22	2.60
Spleen	7.70	11.8	34.50

reusability of the prepared MAA formulations. Best target to non target ration was achieved by using formulation which was stored for 9 days. Consecutive reheating and labeling might conserve heat stable beta form of albumin and results in removal of oxidiazable albumin. The study therefore demonstrates that the formulation of freshly prepared MAA can be stored for 9 days at room temperature and also can be reused effectively and show better target to non target ratio 5min after i.v. administration. The study needs further elaboration in order to increase further the shelf life of laboratory prepared formulation of MAA with reasonably good target to non target ratio.

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