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Research Article

A potential lung perfusion imaging agent of synthetic origin

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Summary

^{99m}Tc-labelled macroaggregated albumin (MAA) is the radiopharmaceutical routinely used for perfusion lung scans. However MAA formulations contain excipients of biological origin, that may potentially cause allergic hypersensitivity in patients. The aim of this study was to prepare a non-biological lung imaging agent, with physiological uptake based on a mechanism of colloid localisation in the pulmonary vasculature. To a frozen stannous fluoride cold kit (RAH Radiopharmacy) was added ^{99m}Tc-pertechnetate ($\leqslant$2 GBq) in saline (1–4 ml), and the radioactive contents were mixed by rotation (40 rpm) in a syringe at room temperature for 30–180 min. The preparative conditions were varied to control particle growth by: the addition of metal ions, halide ions, or oxidants; different mixing times; and temperatures. The ^{99m}Tc products were analysed for % radiolabelling efficiency (RE), radioactive particle size distribution (RPSD), qualitative and quantitative rat biodistribution studies. Results indicated that all radioactive particles were formed with > 99% RE, and 1–47% were > 8 μm. The optimum radiotracer formulation containing the highest proportion of the largest particles, was prepared by mixing SnF_2 and ^{99m}Tc-pertechnetate with a low [Na^+] at room temperature for 50 min. Results from the quantitative organ assays gave 88 $\pm$ 1% tracer in the lungs, and less than 10% in the liver and spleen. The images showed excellent, uniform lung uptake with minimal interference from liver and spleen to the lower regions of right and left lobes. In conclusion, the synthetic radiopharmaceutical ^{99m}Tc–tin fluoride colloid can be prepared with a large particle size, from a commercially available cold kit in a simple and practical manner, and it has good potential for use as a perfusion imaging agent in lung scans.

Key Words: ^{99m}Tc–tin colloid; particle size; lung; MAA

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Introduction

^{99m}Tc-labelled macroaggregated albumin or ^{99m}Tc-Macrosalb (MAA) is the current mainstream radiopharmaceutical used in nuclear medicine for perfusion lung scans. MAA is a suspension of albumin, where the particles are irregular and insoluble aggregates prepared by heat denaturation of the protein in the presence of stannous ions with buffer salts in aqueous solution. Commercial MAA cold kits contain a freeze-dried formulation, where upon reconstitution with ^{99m}Tc-pertechnetate, ^{99m}Tc–MAA is formed over a period of 1–15 min at room temperature. The particles of the suspension have a typical diameter between 10 and 100 μm,[1] mainly 20–50 μm[2] or 15–30 μm.[3] Particles in the ~10 μm range are taken up by the alveolar macrophages, where their opsonisation by endogenous proteins is not essential.[4] Those particles that are greater than 10 μm remain in lung capillaries by a mechanical process akin to 'filtration'. The excipients of the MAA formulation are of biological origin, which results in this product to be contraindicated in persons with a hypersensitivity to human serum albumin.[5]

^{99m}Tc–tin fluoride colloid has been used extensively in Australia to radiolabel leukocytes in whole blood *ex vivo*, of patients with inflammatory bowel disease. The mechanism of labelling the leukocytes is thought to involve phagocytosis of the colloidal particles by macrophages. The ^{99m}Tc–tin fluoride colloid particles used in this procedure are 1–3 μm diameter in size,[6] and are appropriate for phagocytic engulfment, yet too small for significant retention by the pulmonary vasculature. The aim of this study was to investigate the growth phase of ^{99m}Tc–tin fluoride colloid, and to ultimately prepare larger particles of this synthetic agent that is suitable for lung imaging.

Methods

General

Sodium ^{99m}Tc-pertechnetate was obtained from the daily milking of a ^{99}Mo/^{99m}Tc generator (Gentech, Australian Radioisotopes, Sydney, Australia). All solutions employed in the experiments were of pharmaceutical grade and suitable for human use. All experiments were performed in triplicate unless stated otherwise. Results are reported as mean ± standard error.

Radiolabelling procedure

^{99m}Tc–tin fluoride colloid was prepared using LWC Kit [A + B] (RAH Radiopharmacy, Adelaide, Australia). From the Kit, only vial B (stored frozen) containing SnF_2 (0.64 mg) in water for injection (1 ml) was used. The procedure involved adding ^{99m}Tc-pertechnetate (20–1000 MBq) in saline (0.9%; 0.5, 1, 1.5, 4.0 ml) to thawed vial B containing a breather needle, and then the contents were mixed by rotation (~40 rpm) at room temperature

(23°C) for 30–180 min using a rotation apparatus (RSM6 suspension mixer, Ratek Instruments, Victoria, Australia). Experiments were also performed to examine the effects of: (i) nitrogen versus air in the vial, vacuum versus normal atmospheric pressure; (ii) mixing and mixing time; (iii) room temperature (23°C) versus 37°C; and (iv) calcium chloride (0.1 ml; 0.2 ml; 14 mg/ml in WFI), magnesium sulphate (0.1 ml; 49% w/v), sodium hypochlorite (0.06 mg; 1 mg/ml in WFI), excess oxygen gas, sodium bicarbonate (0.1 ml; 8.4% w/v), disodium hydrogen phosphate buffer (0.04 ml; 49% w/v) and sodium fluoride (4.0 ml; 1.25 mg/ml). Radiocolloid samples were used immediately after preparation.

Quality control analyses

Radiochemical purity (RCP) of the radiopharmaceutical was determined by ascending instant thin-layer paper chromatography with normal saline as the eluent. ^{99m}Tc–tin fluoride colloid remained at the origin ($R_f = 0.0$) and ^{99m}Tc-pertechnetate migrated with the solvent front ($R_f = 1.0$). Radioactive paper sections were counted in a gamma counter (Cobra II Auto-Gamma, Canberra Packard, Victoria, Australia) and % RCP was calculated as 100% – % ^{99m}Tc-pertechnetate activity.

Radioactive particle size distribution

All filters were pre-equilibrated with saline (2 ml). Typically, a radiocolloid sample (0.2 ml) was filtered, then the filter was rinsed with saline (2 ml). The filter and filtrates were each counted to determine the % activity above and below the filter size, and these values were used to calculate % RPSD. Filters used were 8, 5 and 3 μm (polycarbonate, Nucleopore).

Quantitative rat biodistributions

The experiments performed with rats complied with 'The Australian Code of Practice for the Care and Use of Animals for Scientific Purposes NHMRC' and according to a protocol approved by the Animal Ethics Committee of the Institute of Medical and Veterinary Science. A pharmacopoeial physiological distribution test[1] for MAA injection was used as a reference to examine the *in vivo* behaviour of 'larger' ^{99m}Tc–tin fluoride colloid particles. Three rats (Sprague–Dawley; female; 130–170 g) were each injected intravenously via the tail vein with ^{99m}Tc–tin fluoride colloid (1–3 MBq; 0.2 ml). After 20 min post-injection (pi) the rats were sacrificed using Halothane asphyxiation, and organs of interest were excised, blotted dry and counted in a large volume counter (Biosentry, AEI-EKCO, Australia) linked to a multichannel analyser (Model 3100, Canberra Industries Inc., USA) over a ^{99m}Tc-window

(70–210 keV). Tails were discarded. All organs were related to the total dose, and the % injected dose/organ (% id) was calculated as a fraction of that total.

Qualitative rat imaging

^{99m}Tc–tin colloid ($\sim$5 MBq; 0.2 ml) was injected intravenously via the tail vein of rats. After 20 min, the animals were sacrificed (Halothane) and then imaged post-mortem on the collimator of a gamma camera (Starcam 4000 XR/T; GE). Static images were acquired for 5 min. The test was repeated using ^{99m}Tc–MAA (triplicate).

Results

Radiochemical purity

The % RCP of ^{99m}Tc–stannous fluoride colloid was found to be 99.4 $\pm$ 0.4% ($n = 3$). The colourless dispersion became opaque during a 3 h observation period.

% RPSD of ^{99m}Tc colloid prepared under different conditions

The effect of the vial inner atmosphere on the particle size of ^{99m}Tc–tin fluoride colloid is shown in Table 1. The parameters kept constant during the preparations were reconstitution volume (saline; 0.9%; 1 ml), rotation speed (40 rpm) and temperature (23°C). For a mixing period of 50 min, an air rather than nitrogen atmosphere resulted in a larger proportion of particles > 5 μm. A partial vacuum versus normal pressure gave similar RPSD for 30 and 50 min mixing periods. The 30 min mixing period resulted in less ($\sim$10%) of the desired larger particles, suggesting that a longer mixing time of 50 min was necessary to obtain $\sim$30% of the population > 8 μm (see also Table 2).

The effect of mixing time on ^{99m}Tc–tin fluoride colloid particle size is summarised in Table 2. The parameters kept constant during the preparations

Table 1. Vial inner atmosphere versus % RPSD of ^{99m}Tc–tin fluoride colloid

	% RPSD		
Atmosphere	> 8 μm	5–8 μm	< 5 μm
Normal pressure/air[a]	33.7 $\pm$ 1.5	46.5 $\pm$ 1.9	19.7 $\pm$ 0.4
Partial vacuum/air[a]	32.6 $\pm$ 2.6	49.3 $\pm$ 2.4	18.2 $\pm$ 0.4
Partial vacuum/N_2[a]	21.7 $\pm$ 0.8	44.9 $\pm$ 1.0	33.3 $\pm$ 0.2
Normal pressure/air[b]	7.1 $\pm$ 0.3	92.9 $\pm$ 0.3[c]	
Partial vacuum/air[b]	10.4 $\pm$ 0.4	36.4 $\pm$ 0.9	53.2 $\pm$ 0.8

[a]Mixing time of 50 min.
[b]Mixing time of 30 min.
[c]% particles < 8 μm.

Table 2. Mixing time of ^{99m}Tc–tin fluoride colloid versus % RPSD

	% RPSD			
Mixing time (min)	> 8 μm	5–8 μm		< 5 μm
30	7.1 ± 0.3		92.9 ± 0.3[b]	
50	33.7 ± 1.5	46.5 ± 1.9		19.7 ± 0.4
180	33.0 ± 2.1		66.7 ± 2.1[b]	
150[a] (static)	47.1 ± 4.4	49.3 ± 4.4		3.6 ± 0.1

[a]Thirty minutes of mixing, then 150 min static at room temperature.
[b]% particles < 8 μm.

Table 3. Temperature of ^{99m}Tc–tin fluoride colloid preparation versus % RPSD

	% RPSD	
Temperature (°C)	> 8 μm	< 8 μm
23	7.1 ± 0.3	92.9 ± 0.3
37	5.6 ± 0.6	94.4 ± 0.6

were saline (1 ml), rotation speed (40 rpm), normal pressure, air atmosphere and temperature (23°C). Results showed a trend that when mixing time increased, the proportion of larger particles (> 8 μm) also increased. When a kit prepared with a 30 min mixing time was left to stand at room temperature for a further 150 min, 4% of radiocolloid particles were < 5 μm. The effect of temperature on the particle size of ^{99m}Tc–tin fluoride colloid is shown in Table 3. The parameters kept constant were saline (1 ml), rotation speed (40 rpm), mixing time (30 min), normal pressure and air atmosphere. As temperature increased the proportion of larger particles (> 8 μm) did not change significantly.

The influence of ionic salts, alkaline buffers and oxidants on the % RPSD of ^{99m}Tc–tin fluoride colloid is shown in Table 4. The parameters kept constant were rotation speed (40 rpm), mixing time (30 min), normal pressure, air atmosphere and temperature (23°C). As the level of sodium chloride increased, a minor increase was observed in the > 8 μm-sized particles. When Ca^{2+} or Mg^{2+} ions were added to the saline dispersion, there was a small increase in the % larger particles, but the majority of particles were < 8 μm. The alkaline salts ($NaHCO_3$, Na_2HPO_4) altered the acidity of the dispersion to pH 8, that in turn resulted in a smaller % of the 8 μm particles, compared to the NaCl series. Sodium fluoride at 5 mg did not influence particle growth significantly, with 9% of particles being > 5 μm. A low level of sodium hypochlorite (60 μg) or an excess of oxygen gas resulted in most of the radioactivity being associated with the smaller tin fluoride particles (< 3 μm for O_2).

Table 4. Influence of ion salts and oxidants on the % RPSD of ^{99m}Tc–tin fluoride colloid

		% RPSD			
Chemical	Mass (mg)	>8 μm	5–8 μm		<5 μm
NaCl	4.5	6.5 ± 0.5		93.5 ± 0.5[a]	
	9	7.1 ± 0.3		92.9 ± 0.3[a]	
	13.5	5.4 ± 0.7		94.6 ± 0.7[a]	
	36	9.2 ± 0.3	15.1 ± 0.7		75.7 ± 0.9
$CaCl_2$	1	3.9 ± 0.5	8.6 ± 0.3		87.5 ± 0.8
	2	6.7 ± 0.4		93.3 ± 0.4[a]	
$MgSO_4$	49	5.2 ± 0.7		94.8 ± 0.7[a]	
NaOCl	0.06	3.0 ± 0.6	3.4 ± 0.4		93.6 ± 0.3
O_2	excess		4.9 ± 0.8[b]		95.1 ± 0.1[c]
$NaHCO_3$	8.4	1.8 ± 0.2		98.2 ± 0.2[a]	
Na_2HPO_4	4.9	1.0 ± 0.0		99.0 ± 0.0[a]	
NaF	5		8.9 ± 0.5[d]		91.1 ± 0.7

[a] <8 μm.
[b] >3 μm.
[c] <3 μm.
[d] >5 μm.

Table 5. Quantitative biodistribution of ^{99m}Tc–SnF_2 colloid in rats at 20 min pi

	% injected dose		
Organ	50 min rotation/air	50 min rotation/N_2	150 min (static)/air
Lungs	88.9 ± 0.8	74.5 ± 0.5	87.8 ± 0.6
Liver	5.7 ± 0.4	13.9 ± 0.3	7.1 ± 0.4
Spleen	0.3 ± 0.0	1.4 ± 0.1	0.7 ± 0.1

Rat physiological distributions

The biodistribution of ^{99m}Tc–tin fluoride colloid prepared by three conditions can be seen in Table 5. More than 70% of ^{99m}Tc–tin fluoride colloid localised in the lungs of rats for the preparative conditions stated, where lower lung activity was found from the kit prepared under a partial vacuum and nitrogen atmosphere (50 min). Similar biodistribution results were obtained for 50 min mixing time versus 30 min mix + 150 min static condition, where lung uptake exceeded 87%, 6–7% was in the liver and trace amounts in the spleen (0.3–0.7%). The images in Figure 1 concur with the quantitative animal data, showing predominant uptake in the lungs, and minor uptake in the liver and

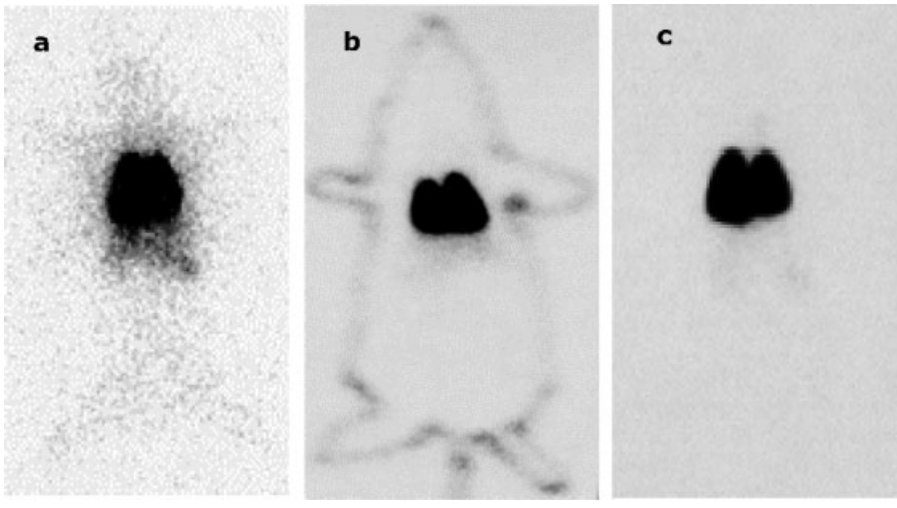

Figure 1. Images of ^{99m}Tc–tin fluoride colloid in rats at 20 min pi prepared by (a) 30 min mix + 150 min static; (b) 50 min mixing time or optimum formulation – rat outline was created with ^{99m}Tc marker during acquisition; and (c) ^{99m}Tc–MAA scan in rats at 20 min pi

spleen. The ^{99m}Tc–tin colloid images did not appear to be significantly different to the ^{99m}Tc–MAA images.

Discussion

^{99m}Tc–tin fluoride colloid is comprised of 1–3 μm size particles when it is used for labelling leukocytes in whole blood. In the preparation of this agent, sodium fluoride (5 mg) is added to stannous fluoride, and then the colourless liquid is filtered (0.2 μm), before an aliquot is added to diluted ^{99m}Tc-pertechnetate. Two processes are occurring simultaneously during this procedure: (i) primary particle formation and (ii) particle growth. The primary or 'template' particles are presumably formed during the mixing phase, then ^{99m}Tc-pertechnetate reduction and chelation of ^{99m}Tc occurs onto the particle surface during the growth phase. The template particles have been reported as having a 0.4 μm diameter,[7] or a mean particle size of 234 $\pm$ 22 nm (Hepatate II; Amerscan) at reconstitution. It is possible that stannous fluoride in the cold kit is partially hydrolysed in the form of mixed particle sizes, where the coarse particles (> 200 nm) are filtered off to give an array of smaller particles up to the size threshold.[6]

The RPSD results in this study suggest that sodium fluoride did not facilitate particle growth. From the data presented here, the exact function of NaF in the preparation of ^{99m}Tc–tin fluoride for leukocyte labelling is not clear. When this halide salt was replaced with sodium chloride, larger particles were formed. Furthermore, with an increase in the level of NaCl, was a trend

indicating a minor increase in the larger particles of ^{99m}Tc–tin fluoride colloid, in agreement with a previous observation.[7] The growth phase of the template particles was not significantly stimulated by calcium chloride or magnesium sulphate, where it was hoped that these divalent cations could initiate aggregation of radiocolloid. The alkaline salts gave a low percentage of $> 8\,\mu m$ particles, that can be explained by the electrical double layer on the colloidal surface being extensively disrupted to give smaller subunits. There was a domination of smaller particles when the oxidants were used, even with a low amount of NaOCl, indicating a successful oxidation of colloidal particles during the growth phase ought to be slow, otherwise deflocculation will ensue.

When nitrogen was present in the vial atmosphere 22% of radioactive particles were $> 8\,\mu m$, or slightly higher (27%) with air. These results confirm that air inside the vial has a minor oxidative effect due to the oxygen gas component, although the main path of oxidation occurs by hydrolysis in aqueous solution. Stannous fluoride is a weak acceptor molecule towards neutral donors such as H_2O, and it is hydrolysed by water alone[8] to give tin oxyfluoride as a general product (Equation (1)). It follows then, that hydrolysis of some stannous fluoride subunits in the colloidal matrix is necessary during particle growth, but total hydrolysis would prevent tin atoms to form other bonds.

$$SnF_2 \leftrightarrow [Sn(OH)F] \rightarrow SnOF_2 \quad (1)$$

The optimum preparative conditions to produce 'larger' ^{99m}Tc–tin fluoride colloid, required an air atmosphere to be inside the vial at normal pressure, ^{99m}Tc-pertechnetate in saline (~ 1 ml), and a mixing time of 50–180 min at 40 rpm at room temperature. A slightly higher percentage of larger particles were found in partial vacuum at 30 min, however this mixing time was too short because there were insufficient $> 8\,\mu m$ ^{99m}Tc–tin colloid particles in comparison to the 50 min mixing period. Where excellent RPSD results and images were obtained at 150 min (static), this mixing period was considered impractical for a busy radiopharmacy that prepares many radiopharmaceutical doses.

A mixing period of 50 min resulted in 34% of radiocolloid particles $> 8\,\mu m$, 47% 5–8 μm, and 20% $< 5\,\mu m$. The quantitative rat distribution of this formulation resulted in a retention of 90% of the injected dose in the lungs and 6% in the liver plus spleen after a 20 min localisation period. These results are quite different to ^{99m}Tc–tin fluoride colloid prepared according to the manufacturer's method for labelling of leukocytes in whole blood, where $> 80\%$ localised in the liver, $< 3\%$ in lungs and $\sim 8\%$ in the spleen of rats.[9] The British Pharmacopoeial quality control specification for the clinical product ^{99m}Tc–MAA is $> 80\%$ in the lungs and less than 5% in the liver plus spleen. The 'larger' ^{99m}Tc–tin fluoride colloid achieves this threshold of lung uptake,

although a low level (< 10%) is also taken up by the liver and spleen. The lung perfusion images suggest that the extent of liver uptake would not limit diagnosis of the lung bases, and they were comparable ^{99m}Tc–MAA scans (Figure 1). Further studies to validate 'larger' ^{99m}Tc–tin fluoride colloid in patients are planned for the near future. This radiocolloid did have surplus large (> 8 μm) ^{99m}Tc particles that were trapped in the pulmonary vasculature. Of the formulation, a significant portion of those particles at or near 5 μm as measured by membrane filtration, were also retained in the lungs as evidenced in the rat images.

In summary, a non-biological ^{99m}Tc–colloid of large particle size has been developed, that is predominantly retained in rat lungs to the extent of ~90%. The low level of liver and spleen uptake (compared to ^{99m}Tc–MAA) is not likely to affect imaging of the lower lung fields, but this needs to be confirmed with further comparative studies. A preparation time of 50 min at present may limit the rapid use of this radiotracer, however a higher ^{99m}Tc-pertechnetate concentration added in the morning could provide multiple lung perfusion doses over the working day.

Conclusion

Based on an investigation concerning the growth phase of ^{99m}Tc–tin fluoride colloid, a lung perfusion imaging agent was developed. This synthetic product is prepared by a simple modification of the leukocyte labelling kit procedure that employs pharmaceutical grade products and a 50 min reconstitution step with ^{99m}Tc-pertechnetate in saline. ^{99m}Tc–tin fluoride colloid prepared in this way has 81% of particles > 5 μm and results in ~90% lung uptake in rats. The results from this work have initiated a study to assess the novel 'larger' ^{99m}Tc–tin fluoride colloid in humans.

References

1. HMSO. *British Pharmacopoeia*, vol. II. HMSO: London, UK, 1993; 1239.
2. MAA Radpharm. Radpharm Scientific Product Information Sheet, 2001.
3. Pulmolite. CIS-US Inc. Product Information Sheet, 2005.
4. Palecanda A, Kobzik L. *Curr Mol Med* 2001; **1**: 589–595.
5. Whinnery JE, Young JT. *J Nucl Med* 1980; **21**: 207–210.
6. Tsopelas C. *ANZSNM News* (*Suppl.*) 2002; **33**: 36.
7. Whateley TL, Steele G. *Eur J Nucl Med* 1985; **10**: 353–357.
8. Nebergall WM, Muhler JC, Day HG. *J Am Chem Soc* 1952; **74**: 1604.
9. Tsopelas C, Smith E, Drew PA, Bartholomeusz FDL. *J Label Compd Radiopharm* 2003; **46**: 751–763.

