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Research paper

Preparation of Tc-99m-macroaggregated albumin from recombinant human albumin for lung perfusion imaging

A.P. Hunt[a], M. Frier[a], R.A. Johnson[b], S. Berezenko[c], A.C. Perkins[a,*]

[a]*Department of Medical Physics, University Hospital, Nottingham, UK*
[b]*Upperton Ltd, Nottingham, UK*
[c]*Delta Biotechnology Ltd, Nottingham, UK*

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Abstract

Human serum albumin (HSA) extracted from pooled blood taken from human donors is used in the production of ^{99m}Tc-labelled macroaggregated albumin (MAA) for lung perfusion imaging. However, concerns for the safety of blood-derived products due to potential contamination by infective agents (e.g. new variant CJD), make alternative production methods necessary. Recombinant DNA technology is a promising method of albumin production avoiding problems associated with human-derived HSA. This paper presents results comparing MAA prepared from recombinant human albumin (rHA, Recombumin®) (rMAA) with in-house produced HSA MAA (hMAA) and commercially available MAA (cMAA). ^{99m}Tc-MAA was prepared using previously published production methods by heating a mixture of albumin and stannous chloride in acetate buffer (pH 5.4) at 70 °C for 20 min. Parameters investigated include aggregate size, radiolabelling efficiency, radiochemical and aggregate stability at 4 °C and in vitro (in whole human blood) at 37 °C and biodistribution studies. Results showed that rMAA could be produced with similar morphology, labelling efficiency and stability to hMAA and cMAA. Our findings confirm that rHA shows significant potential as a direct replacement for HSA in commercially available MAA.
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Keywords: Serum albumin; Macroaggregates; Recombinant albumin; Lung perfusion; Radiopharmaceutical; Lung imaging; Diagnosis of pulmonary embolism

1. Introduction

Ventilation/perfusion (v/2) imaging is widely regarded as the imaging technique of first choice in the diagnostic algorithm for the detection of pulmonary embolism [1,2]. The procedure uses ^{99m}Tc-labelled macroaggregated albumin (^{99m}Tc-MAA) as the imaging agent, with the relatively large MAA particles of the order of 20–40 μm in diameter lodging in the capillary bed of the lung during the first pass of the pulmonary circulation following intravenous administration.

Human serum albumin (HSA) used in the manufacture of ^{99m}Tc-MAA is extracted from the blood of pooled human donors. However, because of the potential health risks associated with HSA (in particular, the threat of contamination by new variant Creutzfeldt Jacob Disease, nvCJD), UK sources have been withdrawn and all HSA medicinal products now use material sourced from outside the UK. Concern is also growing regarding the safety of blood donor materials sourced from other countries. In addition to the UK Department of Health the US Food and Drug administration are monitoring transmissible spongiform encephalopathies from bovine sources [3]. Research has shown that bovine spongiform encephalitis is a member of the sub-acute spongiform encephalopathies caused by transmissible agents including scrapie from sheep and nvCJD in man [4]. Clearly, finding a replacement for HSA in radiopharmacuetical production is of paramount importance to ensure the continued supply of these diagnostic reagents [5].

Recombinant human albumin (rHA) is produced in vitro using recombinant yeast technology, in which genetically modified yeast (*Saccharomyces cerevisiae*) secrete soluble rHA which is subsequently harvested and purified and

* Corresponding author. Department of Medical Physics University Hospital, Nottingham, NG7 2UH, UK, Tel.: +44 115 9709192; fax: +44 115 9422745.
E-mail address: alan.perkins@nottingham.ac.uk (A.C. Perkins).

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formulated for use as an excipient for the manfacture of biologics or a coating for medical devices [6]. The main advantages of rHA over HSA are that it is expressed in yeast with no human derived products used in the manufacturing process. The use of such controlled production methods leads to a purer product with less structural heterogeneity. Previous studies have indicated that there is no significant difference between soluble rHA and HSA in terms of their biochemical characteristics, radiolabelling efficiency and biological behaviour in vitro and in vivo [7]. We have previously demonstrated the concept of using a ^{99m}Tc-rHA microparticle formulation for lung perfusion in an experimental context [8]. The aim of the current work was to develop an MAA product using rHA as a replacement for HSA and to demonstrate equivalence with commercially available MAA products.

2. Materials and methods

2.1. Materials

Clinical grade HSA solution (Zenalb 20), at a concentration of 20% w/v, was obtained from BPL Bioproducts Laboratory, Hertfordshire, UK. Recombinant human albumin solution (Recombumin®) at a concentration of 25% w/v, was supplied by Delta Biotechnology Ltd, Nottingham, UK. Recombumin® is an ultra-high purity product, with residual yeast content of less than 0.15 ppm. Pulmocis MAA kits were purchased from CIS Bio Ltd (Schering Healthcare, Burgess Hill, Sussex, UK). Sodium ^{99m}Tc-pertechnetate solution was supplied by the Radiopharmacy Unit, Queen's Medical Centre, Nottingham, UK. All other chemicals used were purchased from Sigma Aldrich Ltd, Dorset UK.

2.2. In-house preparation of MAA

Two percent rHA or HSA solutions (w/v final concentration) were prepared using pH 5.4, 0.1 M, oxygen-free acetate buffer solution containing 5 mg/ml $SnCl_2$ (w/v final concentration). The solutions were filtered through 0.2 μm polycarbonate membranes into sterile, nitrogen purged polypropylene vials containing magnetic stirrers. The solutions were stirred vigorously for 10 min at room temperature prior to heating in a water bath at 70 °C with constant vigorous stirring for a further 20 min. Following the heating step, the macroaggregate suspensions were cooled immediately in cold water to prevent further aggregation. Soluble albumin was added to the suspensions at a final concentration of 7 mg/ml (w/v) and the preparations were stirred for a final 10 min. Finally, the MAA suspensions were divided into 1 ml aliquots and lyophilised for 24 h. The freeze-dried products were sealed in nitrogen-purged vials and stored in the dark at 4 °C until use (within 2 weeks of production).

2.3. Particle sizing

Twenty microlitre aliquots of the MAA suspensions, recombinant MAA (rMAA), human MAA (hMAA) or commercial MAA (cMAA) were pipetted into a Neubauer Haemocytometer (Hawksley and Sons, Lancing, UK) and viewed using a light microscope (Vickers Instruments, UK) at ×40 final magnification. The longest axis of greater than 100 particles/aliquot was measured using a pre-calibrated eye-piece graticule. Three independent preparations were examined for each formulation.

2.4. Radiolabelling

Vials of lyophilised rMAA and hMAA were reconstituted with sterile saline and washed three times with 0.25 mg/ml ascorbic acid in saline by centrifuging at 1000 rpm for 10 min in a Beckman microcentrifuge. Sodium ^{99m}Tc-pertechnetate solution was added to the washed aggregates to give approximately 100 MBq of ^{99m}Tc to each vial and gently mixed. Preparations were incubated at room temperature for 20 min. Commercial MAA kits (cMAA) were prepared as detailed in the manufacturer's instructions. Labelling efficiency (% LE) was measured by ITLC using SG-ITLC paper (Whatman) and 100% acetone as solvent. Although, the European Pharmacopeia monograph 'Technetium (99m) Macrosalb Injection' [9] uses 3 μm pore polycarbonate filters for determination of non-filtered activity the use of ITLC thin layer chromatography is the standard method adopted for the determination of radiochemical purity within clinical radiopharmacy units in Europe. We have previously validated the ITLC method by comparison with centrifugation to separate bound and unbound ^{99m}Tc products. Following washing with sterile saline to remove unreacted ^{99m}Tc and tin, initial radiochemical purity (% RP) was assessed by SG-ITLC using 100% acetone as solvent. Finally, the stability (% RP) of the three products under storage conditions (4 °C over 6 h) was assessed by ITLC.

2.5. In vitro stability

Aliquots of the ^{99m}Tc-MAA products (0.1 ml) were added to 0.9 ml of whole human blood in heparinised tubes and inverted to mix. The tubes were incubated in a 37 °C water bath throughout the experiment. Aliquots were removed at 0, 0.5, 1, 2, 4, 6 and 24 h and MAA stability was assessed by SG-ITLC using 100% acetone as solvent. Controls for the experiment were: 0.1 ml of ^{99m}Tc pertechnetate in 0.9 ml of whole human blood and 0.1 ml of either ^{99m}Tc-rMAA, ^{99m}Tc-hMAA or ^{99m}Tc-cMAA in 0.9 ml of saline at 37 °C. Replicates from three separate batches of products were performed for all treatments and control experiments.

2.6. Biodistribution study

Approval for the biodistribution study was granted from the UK Home Office and the Biomedical Services Ethics Committee of the University of Nottingham. New Zealand white rabbits were used to investigate the biodistribution and pharmokinetics of rMAA, hMAA and cMAA. Three female rabbits weighing 1.0 kg (approx.) were used, for each formulation. Each product was given as a 1 mg/kg bolus injection into a left ear vein with approximately 10 MBq total radioactivity administered to each animal for rMAA and hMAA formulations and 5 MBq for cMAA. An IGE Maxicamera 400 large field of view gamma camera (IGE, Slough, UK) fitted with a low energy high-resolution collimator was used to image the product distribution over time. Static images were acquired in a 128×128 matrix for 120 s each at 0, 0.5, 1, 2, 5 and 24 h post-dose using a MICAS XNA/P nuclear medicine processing system (Bartec Medical, Farnborough, Hants, UK). Image data were quantified by defining regions of interest (ROI) over the major sites of uptake. At the end of the experiment (24 h) the animals were sacrificed, dissected and the radioactivity in the major organs (lungs, heart, kidneys, liver, bladder) was assessed using the gamma camera. For all images, regions of interest were outlined around the lungs and heart and the other major organs of uptake (thyroid, liver, kidneys and bladder). The count rates from each organ are expressed as a percentage of the whole body counts and all scintigraphic data were corrected for background radiation and radioactive decay.

In addition, 0.5 ml blood samples were taken from the right ear vein at 0, 0.5, 1, 2, 5 and 24 h post-dose and put into heparinised Eppendorf tubes. A 0.1 ml subsample from each blood sample was assayed for whole blood content of ^{99m}Tc in a well scintillation counter (Compugamma, LKB Wallac, Buckinghamshire, UK). The remaining 0.4 ml of each blood sample was centrifuged at 1000 rpm for 5 min to separate cells (pellet) from plasma (supernatant). Each fraction was then placed into separate tubes and counted in a well scintillation counter. A standard was prepared by counting 0.1 ml of the original radiolabelled product in a well scintillation counter. Count rates are expressed as a percentage of the administered activity after correction for decay referenced to the time of dosing.

3. Results

3.1. Particle sizing

rMAA and hMAA had similar morphology to the commercially available CIS/Schering product, although the cMAA did seem to be slightly more condensed than the in-house prepared products (Fig. 1). There was no significant difference in the particle size distribution of the rMAA, hMAA and cMAA (Fig. 2). All preparations had > 90% of the particles within 10–80 μm and no particles > 150 μm were observed. The mean particle size was 29.5 μm (SEM = 0.8 μm), 28.3 μm (SEM = 0.6 μm) and 31.2 μm (SEM = 0.9) for rMAA, hMAA and cMAA, respectively.

3.2. Radiolabelling efficiency, radiochemical purity and in vitro stability

The results showed that both the rMAA and hMAA have comparable radiolabelling efficiency and stability to the commercially available product. Both rMAA and hMAA showed good radiolabelling efficiency with >99% of the ^{99m}Tc associated with the MAA (Table 1). Following centrifugation to remove unreacted ^{99m}Tc, percent radiochemical purity of the products indicated excellent % RP with >99% for all. After storage at 4 °C for 6 h, rMAA still showed >99% radiochemical purity (as did the cMAA) but hMAA had decreased slightly giving >95% RP.

In vitro incubation in whole human blood at 37 °C over 24 h showed no significant differences in product stability over the time course of the experiment between rMAA, hMAA and cMAA (Table 2). After 24 h, >90% of the activity was associated with the product in all cases.

3.3. Biodistribution study

The biodistribution study demonstrated the excellent imaging characteristics of rMAA (Fig. 3): uptake and retention of rMAA in the lungs of the rabbit model was comparable to both the in-house prepared hMAA and the commercially prepared cMAA immediately proceeding administration (Fig. 4). However, the degradation of both in-house prepared products (rMAA and hMAA) was more rapid than the degradation of cMAA as evidenced by

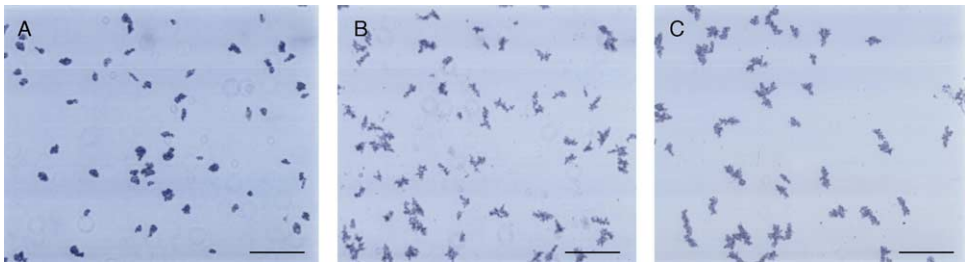

Fig. 1. Photomicrograph of (A) cMAA, (B) hMAA and (C) rMAA. Original magnification ×40. Scale bar is 100 μm.

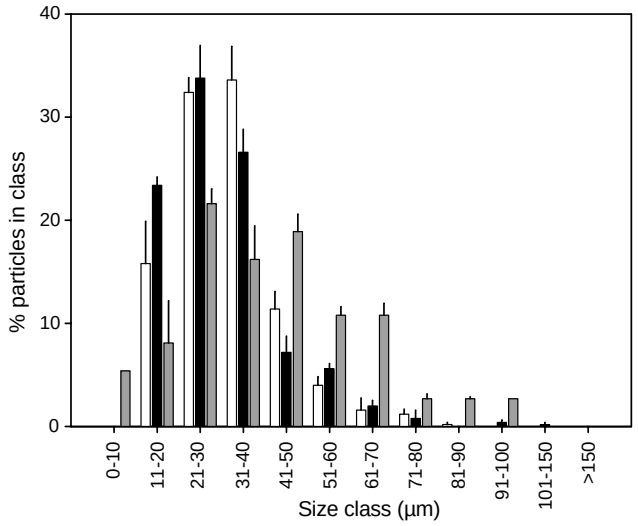

Fig. 2. Size distribution of rMAA (open bars), hMAA (solid black bars) and cMAA (solid grey bars). Vertical lines denote the positive standard error of the mean ($n=3$).

Table 1
Comparison of the percentage labelling efficiency and radiochemical purity (mean(std. dev.)) of rMAA, hMAA and cMAA following labelling with 100 MBq of sodium ^{99m}Tc-pertechnetate (n=3)

Product	Assay		
	% LE	% RP at 4 °C	
RMAA	99.08 (0.58)	99.3 (0.14)	99.14 (0.30)
HMAA	99.89 (0.27)	99.78 (0.07)	95.53 (0.36)
CMAA	99.15 (0.63)	99.24 (0.76)	99.28 (0.24)

LE, labelling efficiency; RP, radiochemical purity.

the increase in activity in the liver and kidneys by 5 h (Figs. 3 and 4).

Data derived from ROIs confirmed the rapid transit of all three MAA products through the heart and >90% of the activity was found in the lungs within a few minutes of administration (Fig. 4). Greater than 80% of the activity remained in the lungs over the first hour of the study but decreased gradually to 20, 34 and 38% for rMAA, hMAA and cMAA, respectively, at 24 h. In the first 2 h after administration, there was no significant difference in the percent administered activity in the lungs and heart for the three preparations for each time point (ANOVA: $0.08 < F > 4.11$, $P > 0.5$). However, at 5 and 24 h post-dose, a significant difference was observed in the activity in the lungs between the three treatments (ANOVA: $F = 20.54$ and 8.83 for 5 and 24 h, respectively) with lower activity in the lungs of subjects treated with rMAA (5 and 24 h, Tukey post hoc test) and hMAA (5 h only, Tukey post hoc test). For all other organs (carcass, liver, kidneys, thyroid, bladder), the sum of the percent-administered dose was less than 45% for the whole of the study period. In the later stages of the study (5–24 h) an increase in activity was observed in the liver, kidneys and bladder for all treatments as the MAA degraded.

Analysis of the radioactivity in whole blood showed that both the rMAA and hMAA followed similar patterns of radiolabel release with differences within 3–4% (Fig. 5). Both rMAA and hMAA showed a rapid, but low level, release of the radiolabel over the study period reaching a maximum of 3 and 2.5% after 1 h for rHA and HSA, respectively. Following the maximum for both treatments, the percentage of the administered dose found in the blood decreased gradually to approximately 1% by 24 h, indicative of the excretion of the ^{99m}Tc from the body. The cMAA showed a similar pattern of release, although lower levels of radiolabel were found in the blood compared to the rMAA and hMAA. The maximum blood activity was observed at 1 h for cMAA when 1.2% of the administered activity was recorded. After the maximum, the mean percent administered activity of cMAA in the blood decreased gradually to approximately 1% of the total administered dose at 24 h.

4. Discussion

Pulmonary embolism is a common potentially fatal condition, for which the correct diagnosis and treatment are highly effective. The clinical diagnosis is challenging and for many years chest radiographs and lung perfusion

Table 2
In vitro stability of MAA formulations expressed as radiochemical purity up to 24 h after radiolabelling (n=3)

Time (h)	Radiochemical purity % (std. dev), treatment						
	rMAA	rMAA control	hMAA	hMAA control	cMAA	cMAA control	Non-specific binding of ^{99m}Tc
0	97.34 (0.34)	97.31 (0.30)	97.86 (0.08)	97.84 (0.18)	96.51 (0.38)	96.28 (0.41)	5.22 (0.36)
0.5	95.67 (1.27)	96.93 (1.25)	92.73 (2.13)	95.99 (1.52)	96.70 (0.30)	95.61 (0.30)	4.59 (1.17)
1.0	95.83 (1.32)	93.70 (1.99)	95.04 (2.04)	94.30 (1.71)	96.18 (0.30)	95.43 (0.31)	3.76 (0.94)
2.0	98.07 (0.58)	90.92 (1.03)	95.08 (1.37)	93.17 (0.67)	96.78 (0.32)	91.44 (0.21)	3.76 (0.94)
4.0	97.00 (1.88)	91.40 (2.15)	96.48 (0.16)	96.19 (0.89)	95.99 (0.69)	94.45 (0.89)	3.64 (0.23)
6.0	94.14 (0.34)	90.67 (2.90)	90.25 (2.68)	98.18 (1.19)	96.50 (0.22)	92.83 (0.52)	3.64 (0.23)
24.0	86.75 (2.10)	88.85 (3.47)	80.23 (0.77)	97.35 (0.24)	91.27 (0.78)	93.56 (0.90)	4.34 (1.69)

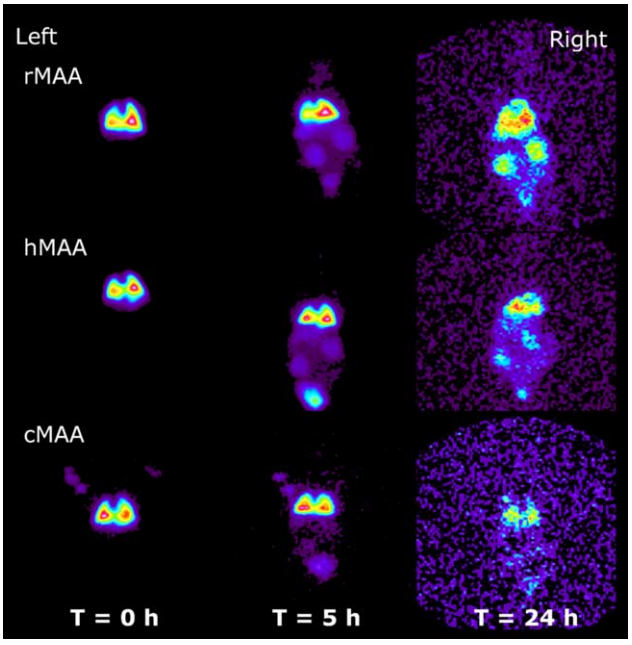

Fig. 3. Static planar gamma camera images (anterior views) illustrating the biodistribution of rMAA, hMAA and cMAA in three representative subjects, 0, 5 and 24 h post-administration. Activity can be seen in the lungs at time zero with clearance via the kidneys and bladder on the later views. The colour scale shows red and yellow as the area with the highest activity with blue indicating lower activity.

imaging has formed the primary diagnostic method [10]. Despite the recent introduction of new imaging techniques for the visualisation of pulmonary embolism such as spiral CT and MRI, V/Q imaging remains an important tool in the diagnostic algorithm. The low cost and rapid patient throughput will ensure that lung perfusion imaging remains of clinical value as a first line diagnostic investigation for the foreseeable future. The additional use of SPECT has been shown to enhance the diagnostic performance of the procedure [11]. It follows, therefore, that in a world context the market for MAA is expected to be sustained for some considerable period.

Pulmonary perfusion imaging relies on one of the simplest methods of tissue localisation used in nuclear imaging, that of capillary blockade of a large particulate in a small microcapillary vessel. Given the current concerns regarding the use of injectable biologicals derived from human blood products the use of HSA as a source material for lung perfusion imaging remains in question. Problems regarding the future use of these materials are unlikely to be resolved and the future prospects are that continued use will become more difficult. It is, therefore, essential that alternative sources of materials are investigated if the procedure is to remain suitable for routine clinical application.

The European Pharmacopeial monograph contains standards relating to the bound radioactivity, particle size (10–100 μm) and biodistribution of the formulation [9]. The present study has addressed and extended the assessment of

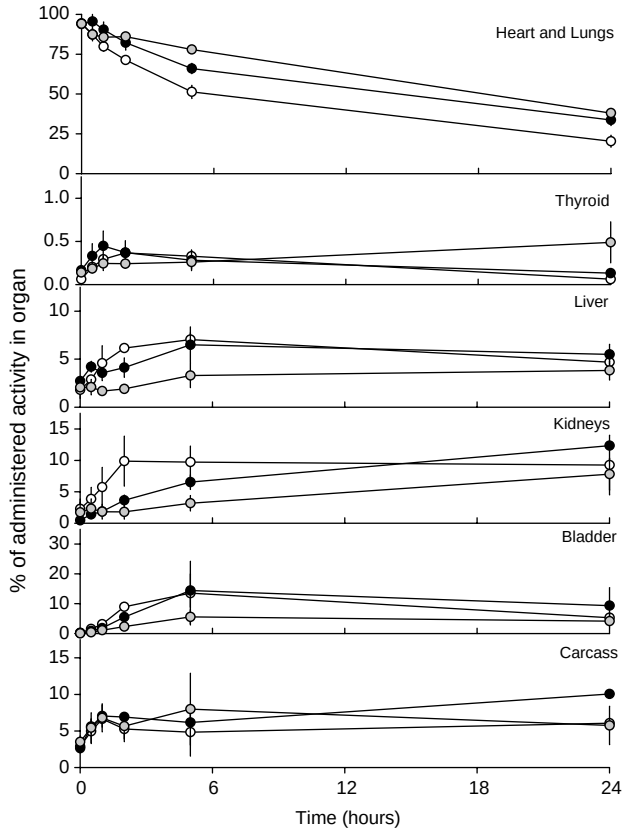

Fig. 4. Comparison of the biodistribution of ^{99m}Tc-MAA in the major organs over 24 h for rMAA (open circles), hMAA (solid black circles) and cMAA (solid grey circles). Vertical lines denote the standard error of the mean ($n=3$).

these characteristics using a commercially available product as a control. We have successfully produced ^{99m}Tc-labelled MAA prepared from rHA and demonstrated operational equivalence by a range of tests comparing with MAA

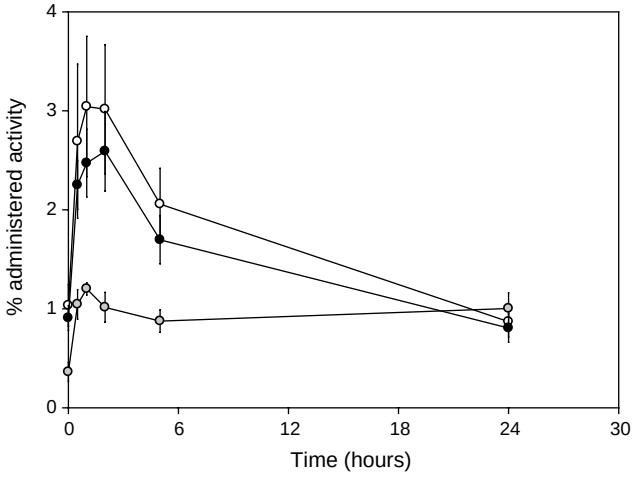

Fig. 5. Percentage of the administered ^{99m}Tc-MAA dose remaining in the blood circulation over 24 h for rMAA (open circles), hMAA (solid black circles) and cMAA (solid grey circles). Vertical lines denote the standard error of the mean ($n=3$).

prepared from HSA and a commercially available product. It is appreciated that differences between commercially and laboratory produced products may occur as a result of the scale of manufacture. It may also be expected that there would be differences between recombinant and natural albumin because the recombinant material is of a higher purity. This study aimed to assess these differences using appropriate controls. The in vitro results demonstrate equivalence in terms of size, morphology and radiolabelling characteristics. In addition, the data show comparable disintegration and clearance of the rHA and MAA, this being an important safety consideration. Furthermore, experimental imaging has shown the ability of ^{99m}Tc-rMAA to localise in lung indicating its suitability for lung perfusion imaging. Use of this material for the detection of pulmonary embolism is still to be demonstrated although the efficacy of the product for this application would seem academic at this stage. Our results show that the rMAA is a viable alternative to the human product. The source material, Recombumin® is now used as a stabiliser in the formulation of protein and peptide therapeutics, vaccines and cell culture media. This represents a valuable alternative for the production of MAA thus ensuring the continued clinical utility of lung perfusion imaging as a first line diagnostic procedure.

Acknowledgements

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