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^{99m}Tc-BRANCHED-CHAIN-POLYPEPTIDE (BCP): A POTENTIAL SYNTHETIC RADIOPHARMACEUTICAL

Alan C Perkins[1], Malcolm Frier[1], Malcolm V Pimm[1,2] and Ferenc Hudecz[3]

[1]Academic Medical Physics, University Hospital, Queen's Medical Centre, Nottingham, NG7 2UH, UK.

[2]Cancer Research Laboratory, University of Nottingham, Nottingham, NG7 2RD

**[3]Research Group for Peptide Chemistry
Hungarian Academy of Sciences
L Eötvös University, Budapest 112
POB 32, Hungary 1518.**

SUMMARY

Synthetic polymers and polypeptides are currently being investigated as a basis for a new generation of radiopharmaceuticals which do not rely on the availability of human blood products or animal derived proteins. In the present study a polyanionic branched chain polypeptide (BCP) based on a poly(L-lysine) backbone with DL-Ala oligomer side chains (three residues on average) terminating in acetylated glutamic acid (AcEAK) was conjugated to DTPA by reaction with DTPA anhydride prior to electrolytic radiolabelling with ^{99m}Tc.

The radiolabelling efficiency of incorporation of ^{99m}Tc was between 40 and 50%. The radiochemical purity on ITLC with butanone was >90%. 10MBq ^{99m}Tc-AcEAK were administered intravenously to normal Wistar rats and the *in vivo* biodistribution assessed by imaging with a gamma camera. Imaging demonstrated the distribution of ^{99m}Tc-AcEAK throughout the body with greatest concentration of activity in the vascular organs such as the heart and liver. Quantification from the images demonstrated 65% of the whole body activity remained at 4 hours. Focal increased uptake was also visualised in the left ear of one rat which was subsequently found to be a site of infection. This study demonstrates that ^{99m}Tc-BCP offers potential for use as a radiopharmaceutical for blood pool and possibly infection imaging.

Keywords: Branched chain polypeptides, ^{99m}Tc, synthetic radiopharmaceuticals.

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INTRODUCTION

Synthetic polymers and polypeptides are an interesting range of materials which when conjugated to drugs demonstrate both increased drug bioavailability and reduced systemic toxicity (1). We have previously radiolabelled various polymers and polymer drug conjugates with gamma emitting radionuclides such as ^{131}I,^{123}I and ^{111}In to enable us to undertake experimental work to examine the biodistribution and tumour targeting properties of these materials (2-6). Imaging studies in normal and tumour-bearing experimental models have provided information on the tumour targeting properties of these compounds and have been valuable tools in the development and understanding of these materials prior to phase I clinical therapeutic trials.

A logical extension of our work was examination of the potential use of these materials as synthetic radiopharmaceuticals. An overriding advantage of these materials is that they offer an alternative to some of the current radiopharmaceuticals which rely on pooled human blood products for their production. Recent concerns over the viral contamination of radiopharmaceuticals derived from human blood products has meant the withdrawal of certain products from clinical use. The use of radiolabelled synthetic polymers and polypeptides as a basis for a new generation of radiopharmaceutical therefore warrants further investigation.

Branched chain polypeptides (BCP) are attractive macromolecules for radiopharmaceuticals since they are water soluble and biodegradable (7). From previous studies using a range of BCPs radiolabelled with ^{111}In we had identified a polyanionic species AcEAK with sustained survival in the circulation following administration to mice with transplanted tumours (8). We have therefore developed a method for radiolabelling this polypeptide with ^{99m}Tc to assess its imaging properties as a blood pool agent in an animal model.

MATERIALS AND METHODS

Radiolabelled Synthetic Branched Chain Polypeptide

The branched chain polypeptide (EAK) was composed of a poly(L-lysine) backbone with DL-alanine oligomer side chains and a terminal glutamic amino acid residue poly[L-Lys-(Glu$_{0.88}$-DL-Ala$_{3.49}$)]. A small proportion of the amino groups of the terminal glutamic acid residue were conjugated to DTPA by reaction with DTPA anhydride at 3:1 molar ratio as previously described (9) and remaining amino groups fully acetylated to give a polyanionic material (AcEAK). The average molecular mass of this material was 60,800 Da. It was selected as a potential blood pool agent from previous biodistribution studies using ^{111}In as the radiolabel (8).

Approximately 5mg AcEAK-DTPA in 0.5ml 0.15M sodium chloride were added to 0.5ml ^{99m}Tc-pertechnetate solution from a molybdenum generator (CIS bioindustry, CIS UK, High

Wycombe). The solution was electrolysed with tin wire electrodes for 2 minutes at a constant current of 2 mA. The solution was then passed through a Sephadex G25 PD10 column (Pharmacia) eluted with 0.15M sodium chloride and 0.5 ml samples collected. The samples were then assayed using a Vinten Isocal II dose calibrator and the two or three (usually samples 7 and 8) containing the first peak ^{99m}Tc were pooled. Ascorbic acid was added to the pooled ^{99m}Tc-AcEAK sample to 0.1mg/ml as anti-oxidant to prevent oxidation of ^{99m}Tc and release of free pertechnetate. The efficiency of radiolabelling was calculated by measuring the percentage of the initial activity contained in the pooled samples and radiochemical purity was measured on ITLC with butanone.

Gel Permeation Chromatography

Gel permeation chromatography of ^{99m}Tc-AcEAK was carried out on a column of Sepharose 4B (Pharmacia) column dimensions being 1.8 x 50 cm, elution being in 0.15 M NaCl containing 1 mg/ml ascorbic acid at a flow rate of 20 ml/hour with automatic collection of fractions of 1.5 ml. The void volume of the column was determined using Blue Dextran (Pharmacea). Radiolabelled polypeptide was applied to the columns diluted into 0.5 ml of rat serum. In addition, runs were carried out with serum prepared from the blood of rats which had been injected 4 hours previously with ^{99m}Tc-AcEAK. Free ^{99m}Tc in the form of pertechnetate added to rat serum was also run through the column.

Biodistribution studies

In vivo biodistribution of ^{99m}Tc-AcEAK preparations was assessed in groups of three normal Wistar rats (Biomedical Services Unit, University of Nottingham) with appropriate licences from the UK Home Office and with due consideration for animal welfare. Approximately 0.5ml of ^{99m}Tc-AcEAK (10MBq) was injected via a tail vein into anaesthetised rats and images acquired using an IGE 400T gamma camera (IGE, Slough UK) fitted with a low energy parallel hole collimator (141keV maximum energy). Views of 120s duration were taken immediately following administration and at hourly intervals up to 4 hours.

Image data were recorded by a nuclear medicine computer in a 64 x 64 matrix and subsequently archived to magnetic tape. The images were then viewed to compare the relative biodistribution at the different time points. Regions of interest were defined to measure the relative activity in the whole body, heart, liver and urinary bladder. Data were expressed as a percentage of the administered dose (decay corrected) at each time point.

RESULTS

The radiolabelling procedure achieved an overall efficiency of ^{99m}Tc incorporation between 40 and 50%, giving specific activities of 25 MBq/mg. The radiochemical purity on ITLC with butanone was >90%. Gel chromatography on sepharose 4B run with ascorbic acid is shown

in Figure 1. There was a small peak of eluted ^{99m}Tc at fraction 21 (i.e. the void volume of the column), possibly representing some aggregated material, but the majority eluted in a broad peak between fractions 30 and 60, with a trailing shoulder partly coincident with the position of elution of free pertechnetate.

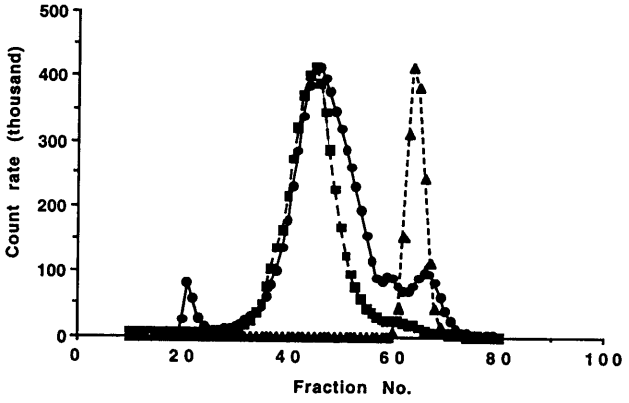

Figure 1. Gel permeation chromatography of ^{99m}Tc-AcEAK on Sepharose 4B in saline with the addition of 1 mg/ml ascorbic acid. Labelled AcEAK added to rat serum (0.5 ml) (●): 100% recovery. Serum (0.5 ml) from a pool collection from three rats four hours after intravenous injection of labelled AcEAK (■): 66% recovery. Free ^{99m}Tc-pertechnetate control (▲) 89% recovery.

Imaging demonstrated the distribution of ^{99m}Tc-AcEAK throughout the body with greatest concentration of activity in the vascular organs such as the heart and liver. Quantification of the pharmacokinetics from the images demonstrated 65% of the whole body activity remained at 4 hours (n=3) (Figure 2).

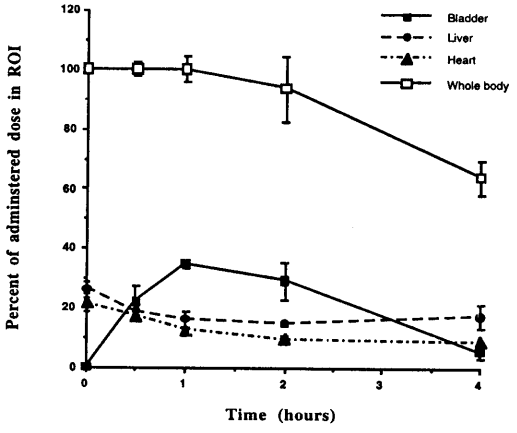

Figure 2. Image quantification of ^{99m}Tc-AcEAK in rats showing whole body, heart, liver and urinary bladder activity (n=3). Data are expressed as a percentage of the administered dose.

This was consistent with the maximum of 35% of the whole body activity which was seen in the urinary bladder at one hour after injection, the majority of which had been voided by 4 hours. Imaging with the gamma camera showed the biodistribution of the conjugate. The organ with the greatest concentration of tracer was the liver which dropped from just under 27% at time 0 to 17% at 4 hours. The activity in the heart dropped from 22% at time 0 to just over 10% at 4 hours. Representative images are shown in Figure 3. Of particular interest was increased uptake in the pinna of the left ear of one rat which was subsequently found to be at a site of infection (Figure 4).

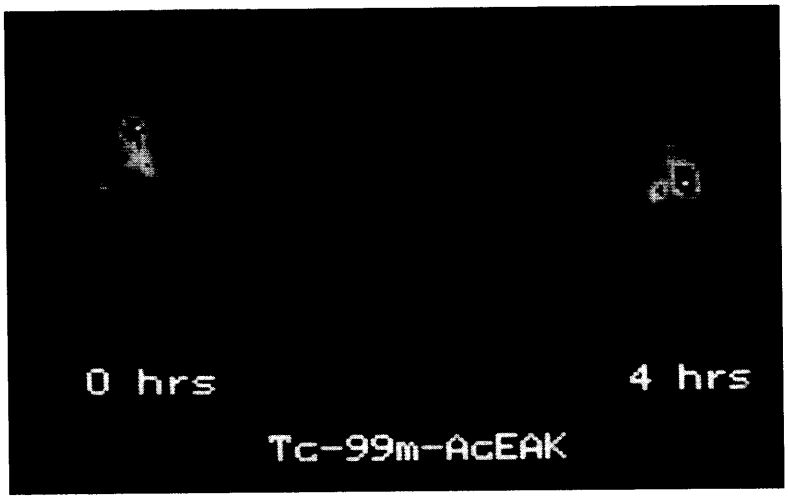

Figure 3. Image of a normal Wistar rat injected with 10MBq ^{99m}Tc-AcEAK: left 0 hours and right 4 hours. Note the whole body distribution of the tracer with greatest concentrations in vascular organs such as the liver and heart.

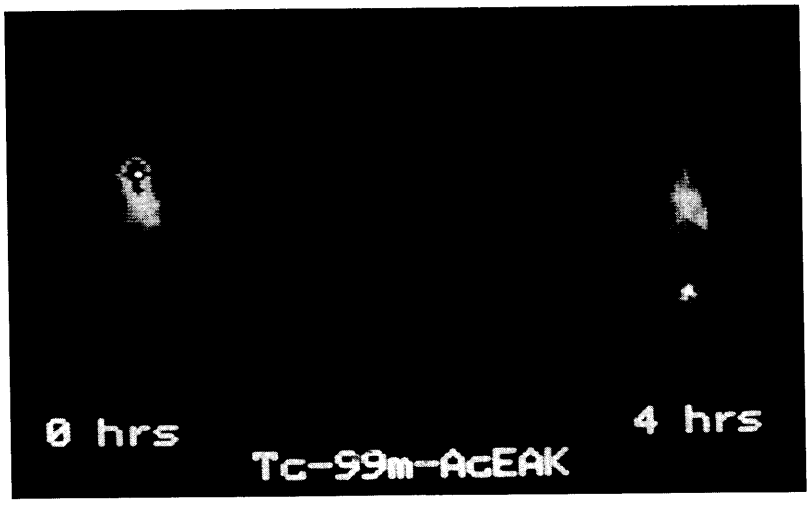

Figure 4. Images of a rat which was found to have an infected left ear pinna: left, 0 hours and right 4 hours after injection of 10MBq ^{99m}Tc-AcEAK. Although tracer distribution is similar to that of the healthy rat shown in Figure 4, increased accumulation can also be seen at the site of infection (left ear) in the 4 hour image.

When serum from the rats injected 4 hours previously with ^{99m}Tc-AcEAK was examined on gel permeation chromatography, the elution profile was similar to that of the injected material, although a single even more discrete peak was seen, the small amount of material eluting at the void volume of the column, the descending trail and material coincident with free pertechnetate no longer being present (Figure 1).

DISCUSSION

Synthetic macromolecules such as polymers and polypeptides are currently being investigated for use in medial diagnosis. In addition to the potential as radiopharmaceuticals these macromolecules are being investigated for use as contrast agents in X-ray, magnetic resonance and ultrasound imaging (10). For example polylysine linked Gd-DTPA has been investigated for its capacity to detect pulmonary perfusion defects in rats using magnetic resonance imaging and polymeric microballoons are being developed for ultrasound imaging (11,12). In nuclear medicine, synthetic polymers have been used to negatively charge-modify monoclonal antibodies, thus altering their *in vivo* biodistribution (3). Our laboratories have previously developed strategies for radiolabelling BCPs with radioiodine (^{123}I,^{125}I and ^{131}I) and radiometals such as ^{111}In and ^{51}Cr for radiopharmaceutical development (4) and we have previously demonstrated the different effects of charge on biodistribution in imaging studies in mice with tumour xenografts (4,8,15). These findings led us to the current studies using ^{99m}Tc which is more widely available and more suited to routine use.

The conjugation of polymers to cytotoxic drugs has been shown to prolong the circulatory survival and reduce systemic toxicity. In previous studies we have demonstrated the imaging characteristics of an N-(2-hydroxypropyl)methacrylamide copolymer-doxorubicin conjugate radiolabelled with either ^{131}I or ^{123}I. Uptake in transplanted melanoma and mammary carcinoma grafts was low (5), however it was possible to show specific targeting to hepatocytes by the conjugation of galactose to the conjugate (6). Similarly, imaging studies using ^{111}In-DTPA-BCP have demonstrated only poor tumour uptake in experimental models but by modification of the side chain structure the charge of the polymer could be altered thus altering the whole body biodistribution and pharmacokinetics (4,8) although not increasing tumour levels.

In the present study we have described a method for radiolabelling this form of BCP polypeptide with ^{99m}Tc. Overall the results from the present study are better than those of Verbeke et. al. (16) who examined ^{99m}Tc-poly-L-lysine as a blood pool imaging agent, but who concluded that it was not suitable for this purpose. In that study the poor vascular survival of the simple form of poly-L-lysine is perhaps not unexpected, since its cationic nature will

result in clearance from the circulation, particularly to the liver and spleen. On the other hand we have used poly(L-lysine) derivatised with short side chains of amino acids (in this case DL-alanine) terminating in glutamic acid (which would render the polypeptide amphoteric rather than cationic under physiological conditions) and which had been acetylated to produce an anionic structure and which we have previously shown to have good survival properties in experimental animals (4,8,15,17). Moreover, an additional feature of the present study was actually to perform imaging in an experimental model. Our results would indicate that although this is not yet an ideal blood pool radiopharmaceutical the heart could be visualised in the early images up to 4 hours and blood pool imaging would indeed be feasible over the first hour, this being the period when, for example, gated cardiac ventriculography is commonly performed. It is also of interest that we have visualised a strong accumulation in a site of infection, raising an additional area for further investigation.

The current problems with the viral contamination of human blood products has placed severe restrictions on the continued production of some radiopharmaceuticals, such as human serum albumin, used for the measurement of plasma volume and macro aggregated albumin (MAA), used for lung perfusion imaging. Recently one of the world's largest commercial suppliers of MAA derived from pooled donor blood, has withdrawn this product due to the possibility of contamination with new variant CJD. It is therefore essential that other reagents are examined for possible future use as radiopharmaceuticals (18). Ultimately these could be formulated as a radiopharmaceutical kit for use in routine clinical practice (19). We have previously proposed synthetic polymers and genetically engineered materials such as recombinant proteins as materials which will allow designer manipulation for the production of radiopharmaceuticals. These results further support the possibility of designer synthetic radiopharmaceuticals based on ^{99m}Tc synthetic branched chain polypeptides. We therefore conclude that these synthetic macromolecules offer potential as valuable alternatives to radiopharmaceuticals produced from human or animal related material offering potential for blood pool imaging and possibly for imaging infection.

Acknowledgements

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