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^{111}In labelling of a branched polypeptide drug carrier with a poly(L-lysine) backbone

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Summary

A synthetic branched polypeptide drug carrier, based on a polylysine backbone with short side chains of three DL-alanine amino acid residues with a terminal glutamic acid residue, has been labelled with ^{111}In by DTPA chelation. ^{111}In is a tracer suitable for gamma scintigraphy, thus broadening the techniques available for examining the biodistribution of such polymeric drug carriers.

Beta-emitting radionuclides can often be incorporated into drug molecules and their biodistribution followed by appropriate counting on blood and tissue samples. The use of gamma emitters would simplify the method and also allow the use of gamma scintigraphy. The major problem here is the labelling of low molecular weight drugs with gamma emitters, particularly to a high enough specific activity for external detection. There is now growing interest in the use of macromolecular carriers for drugs, which may prolong bioavailability of the drug and/or reduce its systemic toxicity (Seymour et al., 1987, 1990; Clegg et al., 1990, 1991). If these macromolecular carrier-drug conjugates could be labelled with an appropriate gamma emitter, scintigraphy would be a useful addition to evaluating their biodistribution.

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Radionuclides suitable for scintigraphy are particularly radioiodines (^{131}I and ^{123}I), and radiometals such as ^{111}In and ^{99m}Tc. Often synthetic macromolecules cannot be labelled with radioiodine in the usual manner, since they lack tyrosine and histidine into which iodine is incorporated by conventional oxidative labelling. One method to overcome this problem is to introduce a small amount of tyrosine into the macromolecular structure, a method used by Seymour et al. (1987) with *N*-(2-hydroxypropyl)methacrylamide copolymers. Similarly, we have previously evaluated the biodistribution in mice of a series of drug carriers based on synthetic polypeptides with a poly(L-lysine) backbone (Hudecz et al., 1985) and labelled with radioiodine by reaction with Bolton and Hunter reagent (*N*-succinimidyl 3-(4-hydroxyphenyl)propionate), prelabelled with ^{125}I, which reacts with α-amino groups of the side-chain terminal amino acids (Clegg et al., 1990, 1991). We report here the labelling of such a synthetic polypeptide drug carrier with radioindium (^{111}In) and blood kinetic and biodistribution studies with it.

The polypeptide used, designated eak, was composed of a polylysine backbone with side chains containing approximately three DL-alanine amino acid residues with a terminal glutamic acid residue and had an average molecular mass of 45 800 Da (Clegg et al., 1990). The polypeptide eak was conjugated to diethylenetriamine-pentaacetic acid (DTPA) by reaction of amino groups on its terminal glutamic acid residues with DTPA anhydride (DTPAA). To an aqueous solution of eak at 2 mg/ml was added dropwise, with continuous stirring, 30 μl of a solution of DTPAA (Sigma Chemical Co., Poole, Dorset, U.K.) in anhydrous dimethyl sulphoxide (DMSO) to give a 3:1 DTPAA:eak ratio. After 10 min at room temperature unreacted DTPA and DMSO were removed and the eak-DTPA transferred to 0.3 M citrate buffer, pH 6.0 by dialysis against four changes of 1000 vols of buffer overnight at 4 °C. The preparation was stored frozen at −20 °C.

For ^{111}In labelling, 15 MBq of ^{111}In as indium chloride (INS1P, Amersham International, Amersham, U.K.) was added to 0.5 mg of the eak-DTPA conjugate in 0.15 ml of pH 6.0 citrate buffer with incubation at room temperature for 10 min before desalting by passage through a 1.5 cm × 5 cm column of Sephadex G-25 with elution in Saline BP. Labelling efficiency was 70–80%. Radiochemical purity as assessed by silica gel thin-layer chromatography, with development in 0.1 M sodium acetate containing 10 mM EDTA, was 95%.

For biodistribution studies Balb/c mice, (Bantin and Kingman, Hull, U.K.) were injected intravenously via a lateral tail vein with 50 μg of ^{111}In labelled eak or approximately the same count rate of free indium chloride in 0.2 ml of saline. Serial blood samples (10 μl) were taken from the tail tip into microcapillary pipettes (Drummond Microcaps, Drummond Scientific Co., Broomhall, PA, U.S.A.) and ^{111}In count rates determined. The labelled material was stable in vivo, having a biodistribution different from that of free ^{111}In. Free ^{111}In will bind to plasma transferrin, and therefore be held in the circulation for some time before undergoing deposition throughout the body and subsequently being slowly excreted. Fig. 1 shows the mean blood clearance

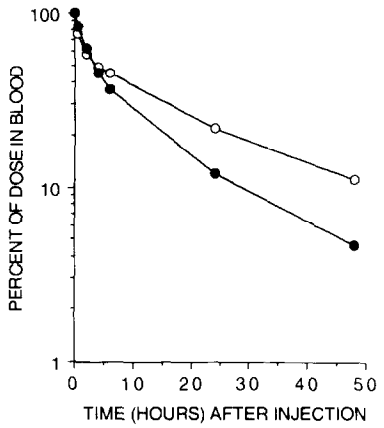

Fig. 1. Blood survival of ^{111}In-labelled eak (○) and free ^{111}-In chloride (●) following intravenous injection into mice. Mean of three mice/group.

profile of ^{111}In labelled eak and free ^{111}In in three mice/group over a 48 h period. The clearance of both materials was essentially biphasic, but they had different clearance rates. Thus, with ^{111}In-eak the first phase (up to 2 h) had a half-time of 2.6 h, the second a half-time of 20.3 h, while with free ^{111}In the first phase, up to 4 h, had a half-time of 3.63 h and a second phase half-time of 14.21 h.

48 h after injection, mice were killed and ^{111}In count rates determined in weighed samples of blood, visceral organs and remaining carcass. The whole body retention of ^{111}In in mice given ^{111}In-eak was 39.6 ± 1.3%, but that of free ^{111}In was 68.2 ± 1.22%. When the ^{111}In count rate/g of each organ of ^{111}In was normalised with respect to the count rate/g of blood there was a marked difference in biodistribution of the two materials (Table 1). Thus, free ^{111}In had tissue to blood

TABLE 1

Biodistribution of ^{111}In-labelled polylysine (eak)

Organ	Tissue:blood ratio (± S.D.)	
	^{111}In-eak	Free ^{111}In
Spleen	0.77 ± 0.02	4.59 ± 1.07
Intestine	0.19 ± 0.01	2.23 ± 0.30
Kidney	4.82 ± 0.67	14.63 ± 3.50
Liver	0.63 ± 0.02	5.44 ± 0.57
Lung	0.39 ± 0.01	2.30 ± 0.12
Carcass	0.27 ± 0.02	2.01 ± 0.42

ratios about 5- or 10-times higher than labelled eak, except in the kidney where it was about 3-fold higher, this organ having the highest ratio with both labelled eak and free ^{111}In.

To assess the nature of circulating radioindium labelled material, gel filtration chromatography was carried out on a 1.8 cm × 90 cm column of Sephacryl-S300 (Pharmacia, Bucks, U.K.) elution being in phosphate-buffered saline at pH 7.3 (PBS) with collection of 2-ml fractions. The elution profile of ^{111}In added to mouse serum was similar to that of the radiolabel persisting in the circulation of mice after 48 h, elution being in the region in which labelled transferrin would be expected to elute from the column. ^{111}In simply added to PBS eluted later, and was clearly distinguishable from the serum-borne ^{111}In (Fig. 2). Labelled eak added to PBS or mouse serum, or in the circulation 48 h after injection all eluted with similar profiles, but clearly distinguishable from free ^{111}In and ^{111}In labelled transferrin. ^{111}In labelled eak in the circulation eluted in a sharper peak, in the apparently highest molecular mass range, and with little (7%) in the region corresponding to labelled transferrin. (It should be noted that although eak had a true average molecular mass of about 46 000 Da it eluted from the column before the labelled transferrin (molecular mass 88 000 Da) in a broad peak centred at about 360 000 Da, and in the region corresponding to the range 150 000–900 000 Da based on calibration with conventional protein standards. This anomaly could be due to the conformation of these molecules, which is known from circular dichroism studies to be relatively unordered (Hudecz et al., 1984, 1988). Similar gel filtration elution profiles are seen with ^{125}I-labelled eak (Clegg, unpublished findings), and thus it is not due to polymerisation by reaction with DTPAA.)

In conclusion, the present study has shown that it is feasible to label this type of polypeptide drug carrier with a tracer suitable for gamma scintigraphy, thus broadening the techniques available for examining their biodistribution, and offering potential for the use of scintigraphy in clinical pharmacokinetic studies.

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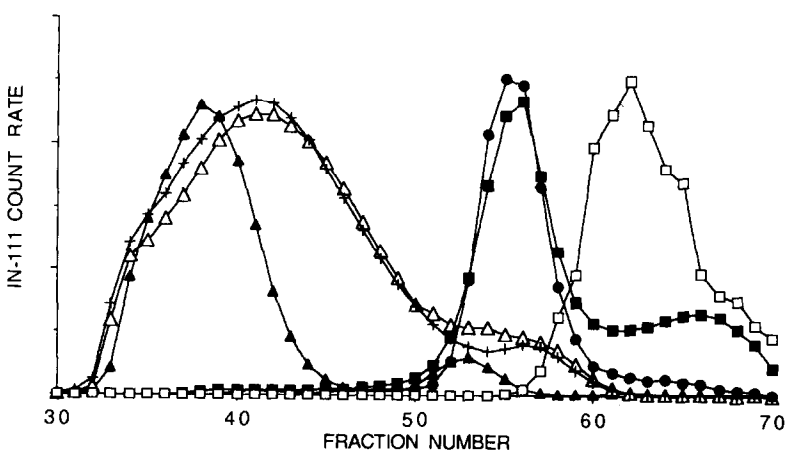

Fig. 2. Gel filtration chromatography on Sephacryl S-300 of ^{111}In-labelled eak and free ^{111}In chloride. (□) ^{111}In in PBS; (■) ^{111}In added to mouse serum; (●) ^{111}In in serum from blood 48 h after injection of ^{111}In; (+) ^{111}In-labelled eak in PBS; (△) ^{111}In labelled eak added to mouse serum; (▲) ^{111}In-labelled eak in serum from blood of mice 48 h after injection of ^{111}In-labelled eak.

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