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Accumulation in the lung of [^{75}Se]norcholestenol administered intravenously in a globular partially quaternized poly[thio-1-(N,N-diethyl-aminomethyl)ethylene] carrier system

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Summary

A novel globular partially quaternized polymer (about 80 Å in diameter) has been used as a carrier system for the intravenous administration of a labelled steroid [^{75}Se]norcholestenol to rabbits. Steroid administered in the free form (ethanol–propylene glycol solution) or contained in an oil-in-water emulsion was used as a control. The distribution of the steroid was followed using gamma-scintigraphy. When entrapped within the globular polymer system, the labelled steroid was taken up to a large extent (50%) in the lungs. This effect was associated with toxic manifestations (the rabbits experienced dypnoea and dystaxia) which were due to an action of the polymer on the microvasculature. Histology showed the pulmonary veins to be engorged with blood; a blockage of the vascular system had taken place. In contrast, when the steroid was administered using a solution and an emulsion formulation, the greater quantity of the observed activity was found in the liver/spleen area (45%) whereas less activity appeared in the lung/heart region (12%).

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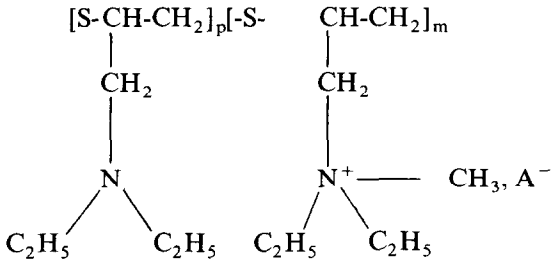
Introduction

Colloidal carriers in the form of liposomes, emulsions, nanoparticles and microspheres have been shown to have potential as drug carriers for selective delivery of drugs to the site of action (Brasseur et al., 1980; Gregoriadis and Allison, 1980; Juliano, 1980). Large particles (greater than 7–10 μm) are usually trapped mechanically in the pulmonary vascular bed while smaller particles normally reach liver and spleen to be captured by cells of the reticuloendothelial system. Particles less than 100 nm in diameter have the possibility of escaping the systemic circulation to reach extravascular sites by passing through the fenestrations of endothelial cells lining the blood vessels of certain tissues, e.g. liver, bone marrow, tumours, etc. (Davis and Illum, 1983). The uptake of particles by phagocytic cells of the liver (Kupffer cells) is normally rapid and efficient but can be influenced by physicochemical factors such as size, surface charge and surface characteristics. The process of particle uptake into phagocytic cells (endocytosis) has been described in detail (De Duve et al., 1974). As part of the sequence of events, the particle can reach the lysosomal apparatus of the cell where it is acted upon by lysosomal enzymes. Some investigators have already suggested taking advantage of the endocytic process and of the low pH in the lysosomes (about 4–5) to obtain intracellular release of drugs temporarily trapped in carriers (Duncan et al., 1983; Gregoriadis, 1981). For example, pH-dependent release of drugs using liposomes (Gregoriadis, 1981) has been reported but efficient systems have yet to be developed.

Recently, polydibasic copolymers of the partially quaternized poly(tertiary amine)-type, Q-P(TDAE)X, have been proposed for entrapment by molecular encapsulation, sustained release and even, pH-induced release of lipophilic drugs (Huguet and Vert, 1985) (Scheme I):



Q-P(TDAE)X

Scheme I with $X = 100 \cdot m/(m+p)$

Indeed, deprotonated Q-P(TDAE)X copolymers (with $X < 20–25\%$) have been shown to form an organic microphase in water composed of macromolecules in a collapsed globular conformation. This microphase can be destabilized by protonation of tertiary amine residues. The macromolecules are easily dispersed in an aqueous environment, and lipophilic materials entrapped within the core of the globules are released over a very narrow pH-range (Vallin et al., 1980).

In a previous paper we have reported that Q-P(TDAE)12 co-polymer injected intramuscularly was able to modify the release of [^{75}Se]norcholestenol, a steroid-type molecule suitably radiolabelled for in vivo monitoring using a gamma-camera. In particular it has been shown that the labelled steroid was entrapped within polymer molecules immobilized in the globular conformation at the injection site. Slow release was obtained, together with a low uptake of the labelled steroid in the liver, as compared to control experiments conducted using a solution of the labelled steroid in ethanol/propylene glycol or a micellar dispersion (Illum et al., 1985).

These promising findings obtained for the intramuscular route, raise the question whether these new globule-forming co-polymers would also be effective for drug delivery and targeting via the intravenous route. The system Q-P(TDAE)12 has potential for intravenous administration on the following grounds: (i) the particles, which are globular macromolecules about 80 Å in size, are smaller than those found for most colloidal carrier systems currently under investigation and could be taken up by conventional phagocytosis as well as by micropinocytosis (coated pits) (Wisse, 1977; Straubinger et al., 1983); (ii) the particles carry a net positive charge which may influence organ distribution, as has already been shown for positively charged microspheres, which are taken up to a greater extent by lungs, than corresponding negatively charged particles (Wilkins and Myers, 1966); and (iii) the pH-dependent conformational transition from globule to extended coil may allow release of entrapped drug in regions of sufficiently low pH (e.g. lysosomes, extracellular regions in tumours, etc).

This paper describes the intravenous administration of the novel polymer Q-P(TDAE)12 using [^{75}Se]norcholestenol as a radiolabelled model drug and the rabbit as an animal model. The fate of the labelled material has been determined non-invasively by gamma-scintigraphy, comparing the polymer–drug system with ethanol/propylene glycol and emulsion systems as controls.

Materials and Methods

Materials

Partially quaternized poly[thio-1-(N,N-diethyl-aminomethyl)ethylene] with 12% N-methylated tertiary amine residues, Q-P(TDAE)12 was prepared and characterized as described before (Vallin et al., 1980). The protonated form thus obtained was converted to the dibasic form (quaternary ammonium hydroxide-tertiary amine) by passing a solution of the salt form through an ion exchange column containing AGl-X4 resin (OH^- form). The polymer system was then partially neutralized by adding suitable amounts of 2 N HCl to convert quaternary ammonium hydroxide residues to their salt form and was made isotonic with sodium chloride. The normality of the solution was 0.155 H^+ equiv./l (i.e. 23 mg/ml, OH^- form) and the pH was 7.7.

[^{75}Se]Norcholestenol was obtained as 100 μg freeze-dried powder (1.08 mCi = 3.4 Ci/mMol) (6-methyl-[^{75}Se]selenomethyl-19-norcholest-5(10)-en-3-ol) from Amersham International. The powder was dissolved in 200 μl ethanol to yield an

ethanolic stock solution of the radioactive material. The [^{75}Se]norcholestenol–Q-P(TDAE)12 system for injection was obtained by mixing 2 ml of the copolymer solution with 40 μl of the ethanolic stock solution of the drug model. Quantitative determination of the extent of trapping of [^{75}Se]norcholestenol in the globular microphase of the polymer was carried out by column chromatography as previously described (Illum et al., 1985); 98% of the steroid was found to be associated with the polymer.

The first control system was prepared by mixing 50 μl of the ethanolic solution with 1 ml of propylene glycol. The second control system consisted of an oil-in-water emulsion system where the radiolabelled compound was contained in the oil phase. The emulsion was prepared using an ultrasonic homogenizer (Dawe Soniprobe) with soybean oil (10%) as the dispersed phase and a mixture (3%) of poloxamers (188 and 388 (2 : 1)) as the emulsifying agent. Glycerol was used to provide isotonicity. The freeze-dried [^{75}Se]norcholestenol was dissolved in the oil phase to give 50 μCi of activity per ml of emulsion. The average particle size was in the region of 500 nm with 95% of the droplets being less than 1 μm in diameter.

In vivo experiments

New Zealand White female rabbits (weight range 2–3 kg) were randomly divided into groups of 3; 0.2 ml of the polymer system, 0.2 ml of the ethanol–propylene glycol solution or 1 ml of the emulsion system, all containing the dissolved labelled steroid, were injected intravenously via the marginal ear vein. The tissue distribution of the polymer system and of the controls was followed for as long as 15 min using external scintigraphic imaging (Maxi Camera II Gamma Camera, International General Electric Company of New York). Dynamic studies (20 s duration) were recorded and processed using a dedicated computer system (GammaScope, Link system).

So-called regions of interest were created around the lungs and liver area, as well as the whole body and the activity therein determined (Illum et al., 1982). The values were corrected for background. The rabbits receiving the polymer system were sacrificed at 5 min and the total radioactivity in the lungs, heart, liver and that remaining in the carcass determined using a large sample volume well-type gamma-counting system (EG and G Ortec).

Results

The activity–time profile for the uptake of [^{75}Se]norcholestenol in the lung/heart and the liver/spleen regions after intravenous administration of the polymer system and the two control systems are shown in Fig. 1. The data have been expressed as a percentage of the total initial activity of the different systems administered. When the [^{75}Se]norcholestenol was administered in the free form (ethanol–propylene glycol solution) or contained in an oil-in-water emulsion, the uptake into the liver/spleen area was quite rapid and sustained and accounted for about 45% of the dose. Both formulations were well tolerated and no apparent toxic effect was

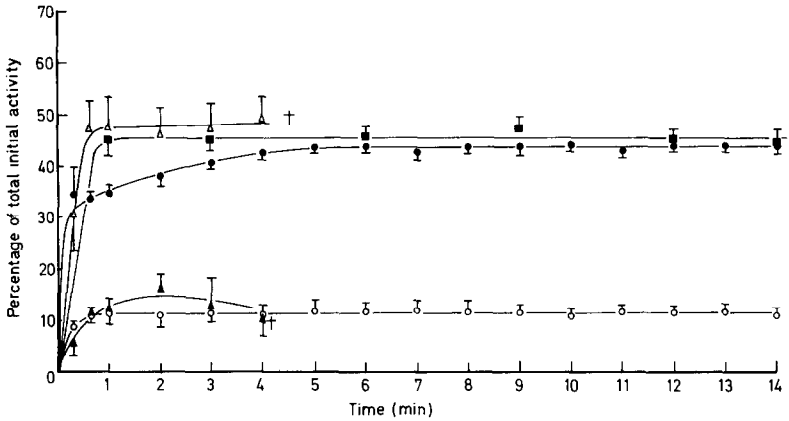


Fig. 1. The percentage of total initial activity of [^{75}Se]norcholestenol in the lung/heart and the liver/spleen regions after intravenous administration of the polymer system and the two control systems. ○, [^{75}Se]norcholestenol in free solution—lung/heart area; ●, [^{75}Se]norcholestenol in free solution—liver/spleen area; △, [^{75}Se]norcholestenol in polymer system—lung/heart area; ▲, [^{75}Se]norcholestenol on polymer system—liver/spleen area; ■, [^{75}Se]norcholestenol in lipid emulsion—liver/spleen area.

detected over an extended period of time (10 days). Besides the liver and spleen no other major sites of accumulation were found. (A representative scintiscan obtained after administration of the ethanol propylene glycol system is shown in Fig. 2A.) The remaining activity was distributed widely in the blood and to other tissue sites. Detailed analysis of the lung/heart regions for the ethanol–propylene glycol and emulsion systems showed a total activity of not more than about 12%.

In contrast, when [^{75}Se]norcholestenol was administered intravenously, entrapped in globular Q-P(TDAE)12 macromolecules, the tissue distribution was very different. Fig. 1 shows that no more than 10–15% of the total initial activity was taken up in the liver/spleen area, whereas 50% was found in the lung/heart region. A typical scintigraphic image is shown in Fig. 2B, demonstrating the pronounced activity in the lung/heart area. The remaining activity was distributed through the body with no other obvious sites of accumulation. These results indicate that when [^{75}Se]norcholestenol is entrapped within the hydrophobic core of the globular Q-P(TDAE)12 polymer, the distribution of the steroid is changed markedly. Unfortunately, the interesting observation of the entrapment of the [^{75}Se]norcholestenol in the globular polymer and its subsequent targeting to the lung/heart area was associated with a gross toxic manifestation. After as little as 4 min after injection the animals experienced difficulties in breathing (dyspnoea, dystaxia) and were sacrificed. The same toxic effect has also been found in rats after intravenous injection of the globular polymer (Bouffard, Huguet, Clabaut, Delpech and Vert, unpublished observations).

On post-mortem examination, the lungs were found to be abnormally inflated. The activity in the isolated organs is shown in Table 1. These data are in good agreement with the scintigraphic information and show that the activity in the

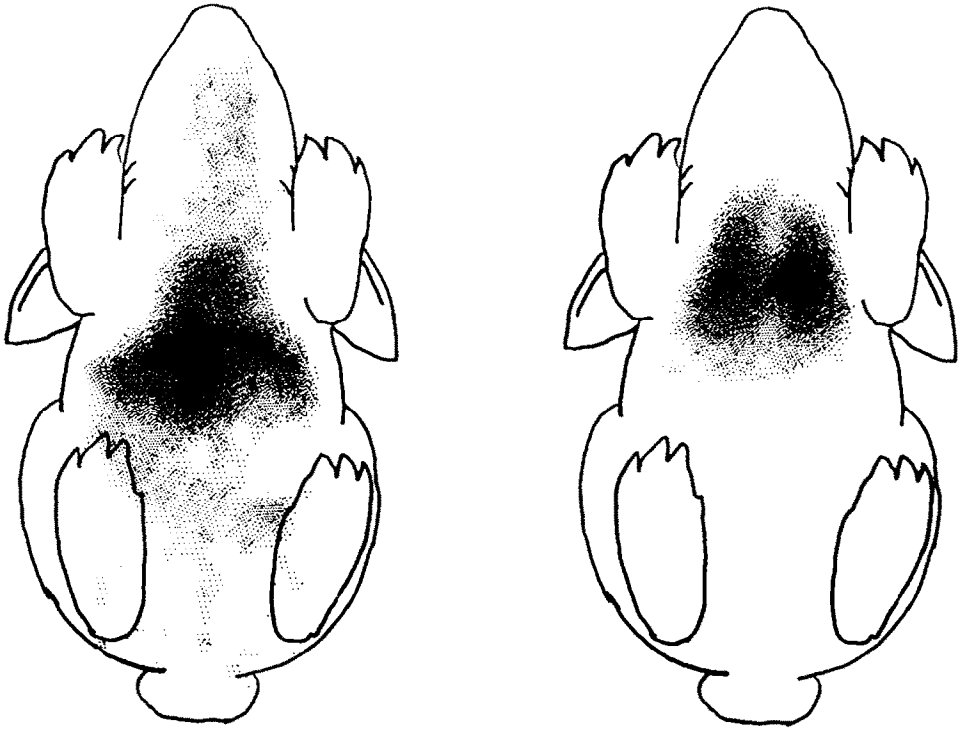


Fig. 2. Scintiscans of rabbits 2–3 min after intravenous injection of [^{75}Se]norcholestenol in: (A) the ethanol–propylene glycol solution; and (B) the polymer system.

lung/heart area is almost completely due to uptake by the lung. Histological examinations showed that the pulmonary capillaries and the branches of the pulmonary veins were engorged with blood. A similar effect was present in the centrolobular veins of the liver and branches of the portal vein. Also evidence of haemorrhage was found in the lungs. These features clearly suggest that a blockage of the vascular system had taken place, most likely in the lungs.

TABLE 1

ORGAN DISTRIBUTION OF [^{75}Se]NORCHOLESTENOL FOLLOWING INTRAVENOUS ADMINISTRATION IN A POLYAMINE (n = 2)

Organ	Percentage of activity
Lung	44.9
Liver	20.7
Heart	1.7
Carcass	32.7

TABLE 2

THE UPTAKE OF [^{75}Se]NORCHOLESTENOL IN THE LIVER FOLLOWING ADMINISTRATION BY DIFFERENT DELIVERY SYSTEMS

Delivery system	Percentage of total/initial activity (%)	Reference
Polymer	15	Present study
Ethanol–propylene glycol solution	45	Present study
Emulsion	45	Present study
Micelles	45	Kreuter et al. (1983)
Polybutylcyanoacrylate/nanoparticles	38	Kreuter et al. (1983)

Discussion

The results obtained for the liver uptake of the labelled steroid following its administration in the two control systems, ethanol–propylene glycol solution and emulsion formulation, are similar to previous data obtained from studies conducted by Kreuter et al. (1983) (Table 2) on [^{75}Se]norcholestenol entrapped in a micellar system and in poly(butylcyanoacrylate) nanoparticles (79.1 nm in diameter) where the liver/spleen uptake was 45% and 38%, respectively.

The uptake of labelled steroid by the liver for all systems listed in Table 2 (except the polymer) is attributed to the rapid phagocytosis of colloidal material by Kupffer cells (Davis and Illum, 1983) as well as to an affinity by the liver for free [^{75}Se]norcholestenol (Nilsson and Zilversmit, 1972). In the case of the colloidal system, the drug is delivered more quickly to the liver than when the drug is in solution, either in ethanol–propylene glycol mixture or in micellar form. It is possible that upon dilution in the blood the [^{75}Se]norcholestenol precipitates out and that the small particles so created are also taken up by the Kupffer cells. We note that for whatever manner in which the material was administered (i.e. emulsion, micelles, nanoparticles or in free solution) the values for liver uptake are all very similar, except for the polymer system (Table 2).

The lung uptake of the [^{75}Se]norcholestenol administered in the polymer system can be explained by three alternative mechanisms: (i) mechanical blockage of the lungs by administered particles; (ii) specific affinity of the positively charged polymer for lung tissue and subsequent toxicity; and (iii) the release of endogenous substances, in particular histamine, leading to an anaphylactoid crisis (vasoconstriction, bronchoconstriction, leading to right-sided heart failure) (Bowman and Rand (1980).

Considering firstly the aspects of blockade, this is thought to be unlikely as far as the globular polymer is concerned. Indeed, the quantity of polymer administered is extremely small, as is the size of the globular (80 Å in diameter) macromolecules. In situ interactions between the polymer and the blood components could result in the formation of aggregates as has been reported for dextran sulphate (Walton, 1954). However, the resultant aggregates should be small in size and therefore be expected to find their way to the liver. The mechanical entrapment of particles in the lungs requires a particle diameter of at least 5–7 μm, that is a growth factor of more than

1000 for the globular polymer molecules. Furthermore, it has been shown previously (Illum et al, 1982; Davis and Taube, 1978) that milligram quantities of colloidal materials in the size range of 40–160 μm can be administered to rabbits without apparent toxicity.

With regard to the second mechanism associated with particle charge, Fidler et al. (1980) have discussed the possibility of directing small particles (< 1 μm) to the lung capillary bed by judicious choice of surface charge and particle size. Indeed, the enhanced uptake of positively charged microspheres by the lungs was reported by Wilkins and Myers (1966). These authors found an initial rapid, appreciable accumulation (15%) of positively charged polystyrene microspheres in the lungs of rats after intravenous administration, followed by a redistribution to the liver and the spleen. Whether such a charge related uptake mechanism is relevant to the present results, is not certain and further work is in progress to develop various positively charged particles and to study organ distribution following intravenous administration (Douglas et al., 1985).

The release of endogenous materials (e.g. histamine) that can then cause gross toxicity is a distinct possibility and could be related to the chemical nature of the polymer. Cationic materials are well known to give rise to toxic effects when administered parenterally. However, in previous studies where the polymer was administered intramuscularly in similar quantities, no obvious toxic effects were observed in rabbits kept for periods of 30 days, or longer, after injection (Illum et al., 1985).

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