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The distribution and fate of ^{131}I-labelled liposomes

J. G. HARDY*, I. W. KELLAWAY**, J. ROGERS† AND C. G. WILSON‡

* *Department of Medical Physics, Queens Medical Centre, University Hospital and Medical School, Nottingham, U.K.,* ** *School of Pharmacy, UWIST, Cardiff, U.K.,* † *Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada,* ‡ *Department of Physiology and Pharmacology, Queens Medical Centre, University Hospital and Medical School, Nottingham, U.K.*

The distribution of ^{131}I-labelled liposomes with sodium iodide entrapped in the aqueous core has been compared with that of a preparation containing iodinated phospholipid, following i.v. administration to rabbits. Liver uptake was observed initially with both liposomes, but the distribution of the former preparation soon became indistinguishable from that of [^{131}I] sodium iodide. Iodine-131 was still detectable in the liver 150 h after administration of the phospholipid labelled material. Scintigrams showing the distributions of the radionuclides following administration of ^{131}I-labelled liposomes and ^{99m}Tc-DTPA liposomes indicate that there may be some biliary clearance.

The labelling of liposomes with suitable γ-emitting radionuclides such as ^{99m}Tc, ^{111}In or ^{131}I enables the distribution of the liposomes to be monitored following the administration of the preparations to animals. By using a γ-camera with an associated computer system, the disposition of the radionuclides can be quantified with minimal disturbance or intervention with normal physiology.

Previously reported studies of liposome distribution have concentrated on the use of technetium-99m, either in the form of pertechnetate ions (McDougall et al 1974, 1975) or as labelled diethylenetriaminepentaacetic acid (^{99m}Tc-DTPA) (Caride et al 1976; Hinkle et al 1978). Following intravenous injection of vesicles containing entrapped pertechnetate into mice, the activity appeared rapidly in the liver, 25% (McDougall et al 1974) and 48% (McDougall et al 1975) being localized within 5 min of dosing. Subcutaneous administration of these preparations resulted in most of the activity remaining at the site of the injection at 1 h, whilst after intraperitoneal injection there was a slow absorption from the site followed by a distribution pattern similar to that observed after intravenous administration (McDougall et al 1975). Following intravenous injection of liposomes containing ^{99m}Tc-DTPA into mice, about 45% of the activity was found in the liver (Caride et al 1976; Hinkle et al 1978). Over 90% of the dose was located in the liver when the liposome diameter was increased from 0·1–2·0 μm to approaching 10 μm. Preloading of both mice and dogs with non-labelled liposomes before injection of the ^{99m}Tc-DTPA-liposomes causes a shift in uptake from the liver to the spleen, kidney, lung and bone (Caride et al 1976).

The 140 keV γ-radiation emitted by ^{99m}Tc is optimum for radionuclide imaging, but the relatively short half-life of 6 h renders it unsuitable for medium term studies. Prolonged studies of the kinetics of disposition and fate of liposomal preparations requires the utilization of radionuclide labels with longer half-lives, for example ^{131}I. This isotope has a half-life of 8·1 days but the higher than optimum γ-radiation of ^{131}I, 364 keV, necessitates the use of a medium energy collimator for the γ-camera. Although this results in slightly reduced resolution, the images obtained provide adequate detail of the radionuclide distribution.

In the present study, the distribution and fate in the rabbit of intravenously administered liposomes containing entrapped [^{131}I]sodium iodide has been compared with liposomes containing ^{131}I covalently bonded to the phospholipid. Both preparations were compared with free [^{131}I]sodium iodide administered by the same route.

MATERIALS AND METHODS

L-α-Dimyristoylphosphatidylcholine (DMPC) (Sigma Chemical Company), dicetylphosphate (DCP) (Sigma Chemical Company) and cholesterol (C) (Fluka, AG. U.S.P. Cryst.) were used as received. Egg phosphatidylcholine (EPC) was purified as described previously (Martin et al 1978). The [^{131}I]sodium iodide solution contained approximately 1 μCi pmol^{-1} iodine. All other chemicals were reagent grade.

‡ Correspondence.

Preparation of liposomes containing entrapped [^{131}I]-sodium iodide

DMPC, C and DCP (5:2:1 molar ratio) were dissolved in chloroform in a 5 ml round-bottom flask and the solvent removed under vacuum at 40 °C to yield 54 mg of a mixed lipid film. The aqueous phase contained 1·25 mCi ml^{-1} [^{131}I]sodium iodide in a pH 7·4 isotonic sodium phosphate buffer (PBS), and 3 ml was added to the flask containing the lipid film. The contents were swirled at 40 °C to disperse the film in the form of polydispersed multilamellar liposomes. The flask was then placed in a 50 kHz ultrasonic bath for 1 h. Untrapped iodide was separated from the liposome dispersion by molecular sieve chromatography. A 2 ml aliquot of the dispersion was delivered via a flow adaptor to a Sephadex-G25 (coarse) column (1·6 × 70 cm) equilibrated in isotonic PBS, pH 7·4 at 5 °C. Fractions of 1·73 ml were collected at a rate of 1·05 ml min^{-1}. The eluent was monitored by flow through an ultraviolet spectrophotometer at 283 nm to detect the liposomal peak, and the level of radioactivity in each fraction was determined using a well counter (Fig. 1). Usually, four fractions at the liposomal peak were pooled to give two samples for immediate injection into the experimental animals.

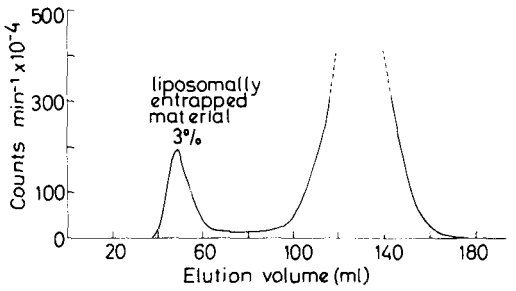

FIG. 1. Separation of liposomally-entrapped [^{131}I]-sodium iodide on a Sephadex G-25 column using isotonic PBS, pH 7·4 at 1·05 ml min^{-1}.

Preparation of liposomes containing entrapped ^{99m}Tc-DTPA

The procedure was similar to that for liposomes containing entrapped sodium iodide, except that the initial activity of the ^{99m}Tc-DTPA was 3·75 mCi ml^{-1}.

Preparation of liposomes labelled with covalently bonded iodine-131

To 1 ml of EPC (5 mg ml^{-1}) in ethanol stirred in a 50 ml round-bottom flask was added the following solutions: 0·25 M PBS (0·2 ml); [^{131}I]sodium iodide, 5 mCi ml^{-1} (1 ml); chloramine-T (Sigma Chemical Company) 5 mg ml^{-1} in 0·05 M PBS, pH 7·5 (0·2 ml) and sodium metabisulphite (1·2 mg ml^{-1}) in 0·05 M PBS, pH 7·5 (0·2 ml).

The solvent was removed under vacuum and EPC, C and DCP were added as solutions in chloroform to produce a lipid mixture of molar ratio EPC:C:DCP (5:2:1). After removing the chloroform under vacuum at 40 °C, 10 ml of distilled water was added to prepare a liposomal suspension containing 36 mg ml^{-1} of lipid which was exhaustively dialysed for 48 h to remove all electrolyte. 10 ml 0·28 M PBS, pH 7·4 was added to 10 ml of the liposomal suspension to yield 20 ml of an isotonic preparation, which was sonicated for 1 h before injection into rabbits.

Characterization of the liposomes

The electrophoretic mobility (−2·08 μm s^{-1} V^{-1} cm^{-1}) of both liposome preparations was measured using a microelectrophoresis apparatus (Rank, Microelectrophoresis Mk. II, Bottisham) with a cylindrical capillary cell at 37 °C.

In vitro radioiodine release rates were determined at 37 °C using a stirred cylindrical dialysis cell with 15 ml donor and receptor compartments separated by dialysis membrane (Spectrapo-1, Spectrum Med. Ind. Inc. Los Angeles, U.S.A.). The liposome suspension (0·25 ml) was mixed with buffer (14·75 ml) in the donor compartment and 0·25 ml samples were withdrawn from the buffer filled receptor compartment over 48 h for radioiodine determinations. Release rates were determined from first order plots and found to be 2% h^{-1} for the [^{131}I]sodium iodide entrapped preparation and 0·15% h^{-1} for the covalently bound ^{131}I preparation. Thin layer chromatography on silica gel plates was carried out on the liposomes containing covalently-bound iodine. X-ray film overlaid on the plate after developing in a chloroform–methanol–water (14:6:1) system showed that the activity was associated with the phospholipid, identified after spraying with Dragendorf's reagent.

The liposomes were sized by Coulter Counter analysis (Model T_A, Coulter Electronics, Harpenden) and the following data obtained using a 50 μm aperture: liposomes with sodium iodide entrapped 14% $\leqslant$ 1·0 μm, 95% $\leqslant$ 2·0 μm; liposomes with covalently bonded iodine 13% $\leqslant$ 1·0 μm, 93% $\leqslant$ 2·0 μm.

Imaging procedure

Doses ranging from 25–250 μCi ^{131}I were administered i.v. into marginal ear veins of the rabbits. The

^{99m}Tc-DTPA doses were 500 μCi when administered in the free state, and 80 μCi when entrapped in the liposomes. Anterior images were taken with the animals lightly restrained in a transparent Perspex box placed on the face of a γ-camera. A medium energy parallel hole collimator was used for the ^{131}I imaging and a low energy parallel hole collimator for the ^{99m}Tc imaging. In each experiment imaging was undertaken at intervals over periods of up to 150 h. Data from the γ-camera were recorded by a computer based data processor. Regions of interest were defined and the radionuclide distributions quantified, the values being corrected for background activity and radioactive decay.

Six animals were dosed with the ^{131}I-phospholipid liposomes, seven with the liposomes containing the entrapped [^{131}I] iodide, and seven with [^{131}I] sodium iodide solution. Each rabbit was used for one experiment only.

RESULTS AND DISCUSSION

Whole body elimination

The elimination of the ^{131}I from the body was followed by whole body counting using the γ-camera. The results for the three preparations are shown in Fig. 2, all the values have been corrected for radioactive decay and background activity. The kinetics of elimination of ^{131}I from all the preparations follow a multiexponential pattern with a rapid phase for the first 40–60 h. The later slower phase largely follows the turnover of iodine in the thyroid, except for the animals which received the iodinated phospholipid liposomes, in which the liver turnover also contributed (Fig. 3). The elimination of iodide-containing liposomes could not be followed for more than 60 h due to the low count rates by this time.

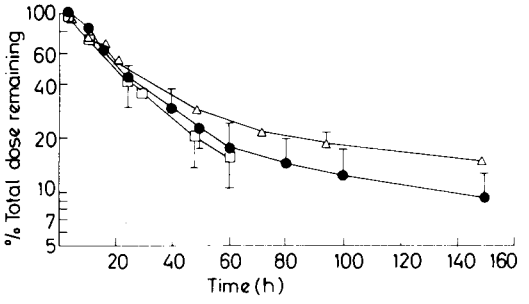

FIG. 2. Whole body elimination of ^{131}I following i.v. administration to rabbits of [^{131}I]sodium iodide in 0·9% NaCl (saline) (●), [^{131}I]sodium iodide entrapped in liposomes (□) and ^{131}I-phospholipid liposomes (△). (Mean shown + or − 1 s.e.).

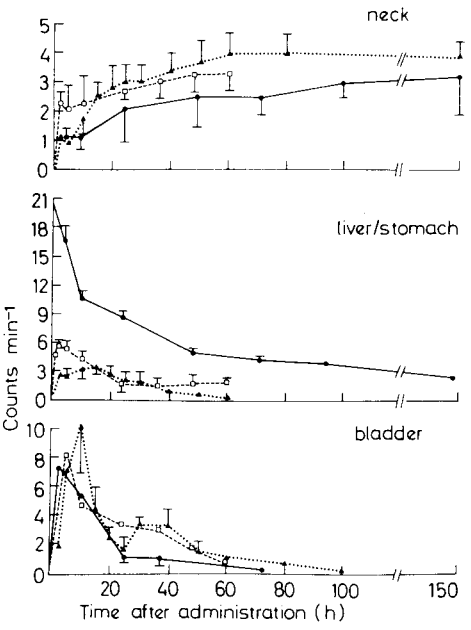

FIG. 3. Distribution of ^{131}I in the neck, liver/stomach, and bladder regions following intravenous administration of [^{131}I]sodium iodide in saline (▲), [^{131}I]sodium iodide entrapped in liposomes (□) and ^{131}I-phospholipid liposomes (●). (Mean shown ± 1 s.e.).

Distribution of the marker

Three areas could be discriminated clearly in scintigrams of the rabbit following administration of the preparations. These were neck, liver/stomach and bladder regions and the radionuclide distributions in these areas were followed throughout the study. The results are shown in Fig. 3.

A gradual accumulation of ^{131}I in the neck region was seen with a small notch in the curve, 1–2 h after administration of free iodide and the liposomally-entrapped iodide. From scintigrams taken during the study, it was seen that the salivary glands accumulated the marker which was then cleared, presumably by swallowing. This behaviour was not discernable in rabbits which received the ^{131}I-phospholipid liposomes. The slowest rate of uptake of iodide by the thyroid was observed following administration of this latter preparation. The release of ^{131}I from the liposomally entrapped iodide appeared to be higher in vivo than in vitro. The thyroid uptake of ^{131}I provided a measure of its release from the preparations. On the termination of the study (150 h), 3·86 ± 0·69% of the dose administered (mean ± 1 s.e.) remained in the neck region of the rabbits (n = 7) which received the iodide, compared with

3·25 ± 1·39% of the dose in animals which received the ^{131}I-phospholipid liposomes (n = 6). The distribution of [^{131}I]sodium iodide in the rabbit is essentially the same as in man, although there are quantitative differences; for example the maximum uptake by the thyroid is much lower in the rabbit (Wong & Schultz 1977; Hardy et al 1978).

Maximum uptake of ^{131}I in the stomachs of the animals which received isotonic sodium iodide solution was 3·14 ± 0·70% at 15 h after dosing (Fig. 3). Within an hour of administration of the ^{131}I phospholipid liposomes 20·52 ± 0·52% of the dose was concentrated in the liver area. Administration of [^{131}I]sodium iodide entrapped within the aqueous core of the liposomes modified the initial distribution of the activity. The liver uptake of the liposomes caused an increase in the amount of activity in the liver/stomach region when compared with the free iodide; the maximum value of 5·65 ± 0·84% being attained 2 h after dosing (n = 7). The preferential uptake of unbound iodide by the stomach of lipid material by the liver, and the overlap of the organs and the images, made it impossible to assign the relative proportions of the dose to the individual organs following administration of the liposomally-entrapped iodide. At 150 h after the administration of the ^{131}I-phospholipid liposomes, 2·25 ± 0·44% of the dose remained in the liver and this could be seen clearly in the scintigram (Fig. 4).

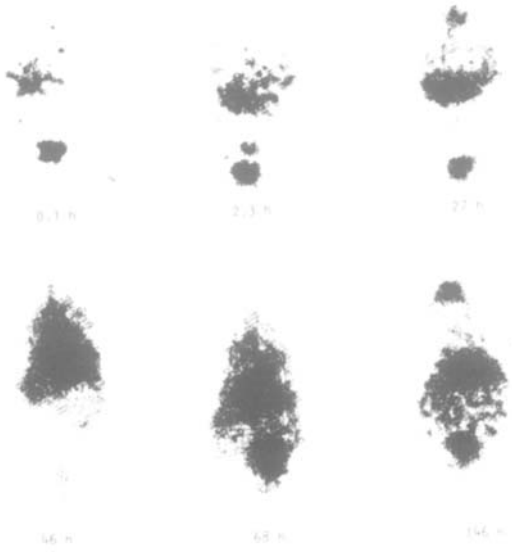

FIG. 4. Scintigrams showing the distribution of ^{131}I following i.v. administration of ^{131}I-phospholipid liposomes to a rabbit.

Appearance of the ^{131}I label in the bladder occurred soon after administration of the preparations. A rhythm in the micturation frequency with periodicity of about a day was evident in animals which received the sodium iodide solution (Fig. 3).

In some of the animals that received ^{131}I-labelled liposomes the scintigrams indicated the presence of the radionuclide in the gastrointestinal tract a few hours after administration (Fig. 4). It was not clear whether the label had originated as a result of ^{131}I containing stomach secretions and saliva, or elimination of the label from the liver in the bile. Accordingly, further studies were carried out using ^{99m}Tc-DTPA, an agent which normally does not appear in the gastrointestinal tract after i.v. administration of isotonic solutions. The rabbits first received ^{99m}Tc-DTPA solution by i.v. injection. The results are shown in Fig. 5a and illustrated that most of the

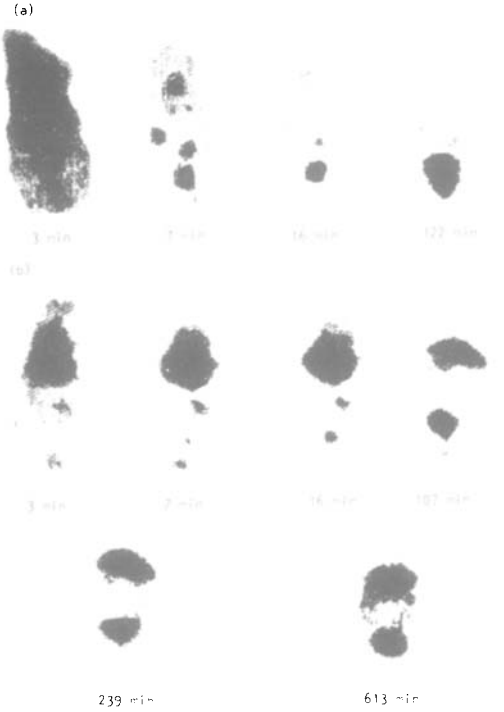

FIG. 5. Scintigrams showing the distribution of ^{99m}Tc-DTPA in the rabbit following i.v. administration of (a) isotonic solution in saline and (b) liposomally-entrapped material.

marker had been cleared by the kidneys at 120 min. Following i.v. administration of the liposomes containing ^{99m}Tc-DTPA, the distribution of the radionuclide and the kinetics of clearance were clearly different. A proportion was cleared by the kidneys but the major fraction of the dose was localized in the liver (Fig. 5b). Images taken 4 and 10 h after

administration show activity which may have been in the intestines, implying possible biliary excretion of either the intact liposomes or release contents. ^{99m}Tc-DTPA is not absorbed from the gut following oral administration to rabbits (Curt et al 1978).

This study has shown conclusively that the ^{131}I formulated in liposomes (a) containing [^{131}I]sodium iodide in the aqueous core and (b) containing covalently bonded ^{131}I in the lipid bilayer exhibited markedly different distributions. In the former case, the [^{131}I]iodide was rapidly released from the liposome and hence the observed distribution was, within a short time, indistinguishable from free iodide. One of the postulated mechanisms by which liposomes undergo cellular uptake is by fusion with the plasmalemma, thus releasing the aqueous core associated with the outermost compartment into the cytosol, whilst the remaining aqueous compartments remain entrapped within the liposome inside the cell (Finkelstein & Weissman 1978). Sessa & Weissmann (1968) have calculated that 10% of the total trapped volume may be released in this way, which might in part explain the discrepancy between the in vitro release rates and the behaviour observed in vivo. In addition, the in vitro tests were undertaken in buffer and not plasma or serum which may render the liposomes more permeable to the marker (Espinola et al 1979).

It is not clear to what extent the ^{131}I when covalently bonded to phospholipid, represents the fate of the liposome. If the entrapped liposome is degraded in situ then the ^{131}I label may be incorporated into other molecules. The intact liposome might also undergo secondary fusion events with intracellular membranes such as the nuclear or lysosomal envelopes (Finkelstein & Weissman 1978). DeBarsy et al (1975, 1976) have demonstrated the presence of liposomes containing fluorescein-conjugated antibodies in the lumen of the sinusoids and within the Kupfer cells which become grossly vacuolated. Parenchymal cells also accumulated the liposomal marker, although this happened after Kupfer cell uptake, and has been shown by Tanaka et al (1975) to be quantitatively less important on a tissue mass basis.

The two liposomal preparations investigated in the present study had similar particle size compositions, although by using a Coulter counter the complete size range is not determined, merely the upper end of the distributions. Variations in size may therefore exist in the lower end of the distribution, although it is unlikely that such variations if present were responsible for the differences observed between the distributions of the covalently bound ^{131}I preparations and the ^{131}I entrapped materials.

Juliano & Stamp (1975) demonstrated that liposomal charge was an important determinant of clearance, hence in this study the charge was examined by micro-electrophoresis. The identical negative micro-electrophoretic mobility for both liposome preparations indicated that the difference in clearance rates between preparations was not attributable to charge effects.

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