DRUG ENTRAPMENT IN LIPOSOMES

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1. Introduction

Treatment of human disorders by the direct administration of drugs can be hampered by immunological reactions, development of drug resistance, uptake of drugs by non-diseased tissues often leading to serious side effects, or even by the inability of some drugs to penetrate target cells. A general carrier within which drugs could be entrapped and directed to target tissues might help to eliminate some of these problems and it has been suggested that the need for such a carrier could be satisfied by liposomes [1, 2]. Liposomes are spherules formed when phospholipids are allowed to swell in aqueous media and they consist of concentric lipid bilayers alternating with aqueous compartments. Within the aqueous or lipid phase of liposomes, water or lipid-soluble substances respectively can be entrapped [3]. Work with enzyme-containing liposomes injected into rats has shown that liposomes can carry enzymes into the liver and spleen [1, 4, 5].

In this report the possibility of employing liposomes as drug carriers is examined. The choice of drugs for entrapment studies included actinomycin D because of its deleterious effect on non-diseased rapidly dividing cells (i.e. intestinal wall) in cancer chemotherapy [6] and penicillin because of its failure to penetrate cells of the reticuloendothelial system (i.e. spleen) in antimicrobial therapy [6]. Experiments with liposomes-entrapped and non-entrapped drugs injected into rats revealed that entrapment directed to a considerable extent the rate of drug elimination from the plasma and drug tissue distribution.

2. Materials and methods

The sources and grades of egg lecithin, cholesterol and phosphatidic acid have been described elsewhere [1, 4, 5]. Synthetic β-γ-dipalmitoyl D, L-α-glyceryl phosphoryl choline (dipalmitoyl lecithin), grade I was purchased from Sigma (London) U.K., and stearylamine (octadecylamine) from K and K Inc., N.Y., U.S.A. Potassium benzyl penicillin was from Dista Products Ltd., Liverpool, U.K., and actinomycin D from Merck, Sharp and Dohme, Pa., U.S.A. Potassium [^{14}C]benzyl penicillin (potassium 6-phenyl [acet-1-^{14}C]amido-penicillanate, 38.2 mCi/mmol), and [^{3}H]actinomycin D (3.7 Ci/mmol) were from The Radiochemical Centre, Amersham, U.K. and ^{125}I-labelled human serum albumin (1 μCi/mg) was kindly donated by Dr. G. Smith of this Centre. For entrapment [1, 4, 5] of drugs mixed with their labelled derivatives in liposomes, lipids (see table 1) were dissolved in $CHCl_3$ and after evaporation under reduced pressure the lipid layer on the walls of the flask was dispersed (at 60–70°C when dipalmitoyl lecithin was present) with 2 ml water containing the drugs (see table 1). Actinomycin D was also entrapped in the lipid phase of liposomes by dissolving the drug and lipids in $CHCl_3$ and dispersing the lipid layer after evaporation of the solvent with 1% NaCl. Four to six hours after their sonication [5], liposomal suspensions were passed through a Sepharose 6B column (Pharmacia) to separate drug-containing liposomes from the non-entrapped drug. In some experiments [^{125}I]albumin was entrapped in liposomes composed of dipalmitoyl lecithin, cholesterol and phosphatidic acid or egg lecithin, cholesterol and stearylamine [1]. Entrapment

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Table 1
Drug entrapment in liposomes.

Lipids	% Entrapped*	
	Penicillin	Actinomycin D
DPL, CHOL, STEAR	5.4	11.0
	6.0	11.6
	4.5	
EL, CHOL, STEAR	5.6	8.8
	7.2	8.5†
		9.2†
DPL, CHOL, PA	8.4	7.0
	3.9	6.8
	7.5	
EL, CHOL, PA	3.2	2.3
	2.2	3.3
	2.3	5.1†
		5.3†

Potassium [^{14}C]benzyl penicillin (10 mg and 5–29 μCi) and [^{3}H]actinomycin D (0.05–0.10 mg and 15 μCi) were entrapped in liposomes composed of 15 mg dipalmitoyl lecithin (DPL) or egg lecithin (EL) each supplemented with 2.2 mg cholesterol (CHOL) and 0.81 mg stearylamine (STEAR) or 2.12 mg phosphatidic acid (PA) (see the Materials and methods section). The molar ratio for each set of phospholipid, cholesterol and charged lipid was 7:2:1.
Entrapment of drugs in liposomes prepared in the absence of charged lipids was negligible.

* % of the radioactivity of the drug used associated with liposomes following molecular sieve chromatography.
† Drug entrapped in the lipid phase of liposomes.

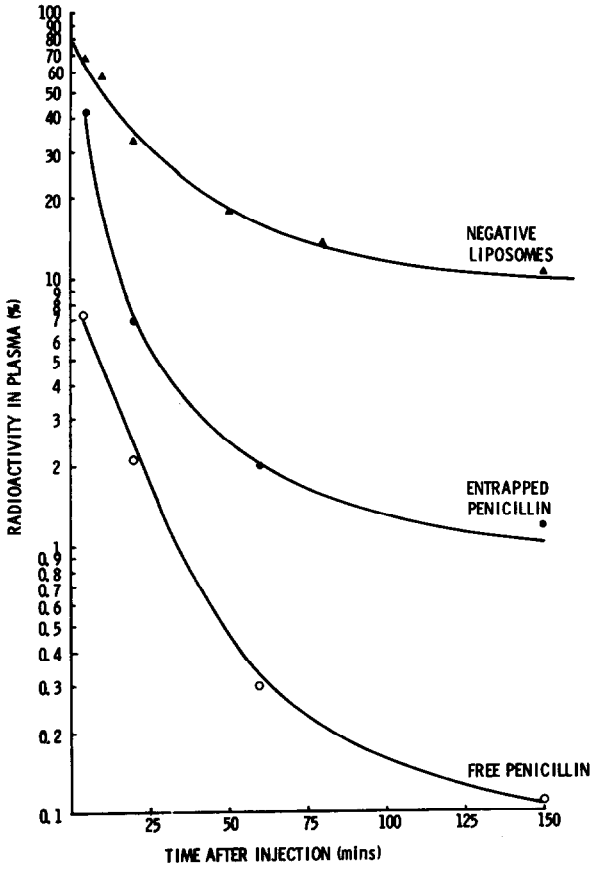

Fig. 1. Elimination from the blood of non-entrapped and liposome-entrapped potassium [^{14}C]benzyl penicillin. Rats were injected with 28 μg (9×10^5 dpm) non-entrapped potassium benzyl penicillin (○—○—○) 30 μg (8.2×10^5 dpm) liposome-entrapped potassium benzyl penicillin (●—●—●) or 0.31 mg (7.2×10^4 cpm) liposome-entrapped [^{125}I]albumin (▲—▲—▲). Liposomes (2.5 mg lipid) were composed of dipalmitoyl lecithin, cholesterol and phosphatidic acid (see legend to table 1). Owing to slow loss of entrapped drugs through diffusion on standing, liposomes were used within hours following their preparation. No such loss was observed with liposomes containing actinomycin D in their lipid phase 45 days after entrapment. At time intervals radioactivity was measured in the plasma the volume of which was taken as 9.8 ml/100 g body weight [5]. Each point % of the radioactivity injected is the average value from 2 to 3 rats.

of albumin in stearylamine liposomes was carried out in 0.1 M acetate buffer pH 4.5.

Male rats (Wistar) weighing 100–150 g were injected in their tail vein with 1 ml of appropriate solutions of labelled non-entrapped drugs in 1% NaCl or with labelled drugs or albumin previously entrapped in liposomes, and decapitated at time intervals. Radioactivity was measured [5] in the plasma and the homogenates [5] of several tissues.

3. Results and discussion

Liposomes were prepared using a phospholipid (dipalmitoyl or egg lecithin), cholesterol and either stearylamine (positive liposomes) or phosphatidic acid (negative liposomes) in a molar ratio of 7:2:1 [1]. Dipalmitoyl lecithin was used because the absence of double bonds in its paraffin chains enhances solute entrapment [7] which is also enhanced by cholesterol

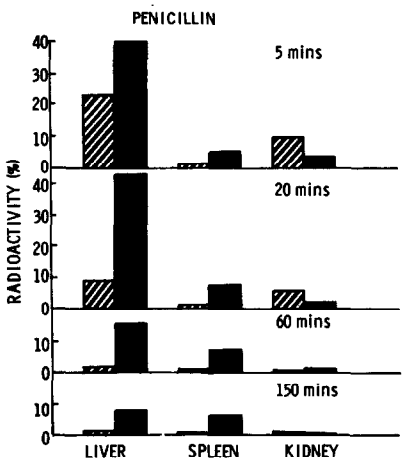

Fig. 2. Time course of uptake and persistence of potassium [^{14}C]benzyl penicillin in rat tissues. Rats were injected with non-entrapped or liposome-entrapped potassium benzyl penicillin as described in fig. 1. At time intervals radioactivity was measured in the tissue homogenates and, when appropriate, corrected for blood contamination. The height of each bar (% of the injected radioactivity per whole tissue) is the average value from 2 to 3 rats. Hatched bars: non-entrapped drugs; filled bars: liposome-entrapped drugs.

[8]. Charged lipids were used because they increase the volume of aqueous spaces within the liposomes, hence the amount of entrapped solutes [9], and they delay diffusion of entrapped ions of homologous charge [10]. It is apparent from table 1 that values for penicillin and actinomycin D entrapment (% of the drug used) in liposomes containing dipalmitoyl lecithin or stearylamine in their structure are, for each drug, higher than those observed with egg lecithin-phosphatidic acid liposomes. This could be attributed to the superiority of dipalmitoyl lecithin over egg lecithin in enhancing solute entrapment [7] and, for at least the weakly basic actinomycin D, to an homologous charge effect [10].

In preliminary experiments non-entrapped drugs and drugs or [^{125}I]albumin entrapped in liposomes of lipid composition shown in table 1 were injected into rats. It was found that 5 min after injection plasma concentration values (% of the injected dose) of entrapped drugs, depending on the lipid composition of the carrier, varied between those for the corresponding non-entrapped drugs (1–7%) and those for liposome-entrapped albumin (60–70% of the dose). Penicillin- or actinomycin D-containing liposomal preparations that exhibited drug plasma values approaching those for entrapped albumin (figs. 1 and 3) as well as non-entrapped drugs were injected into rats and their fate investigated.

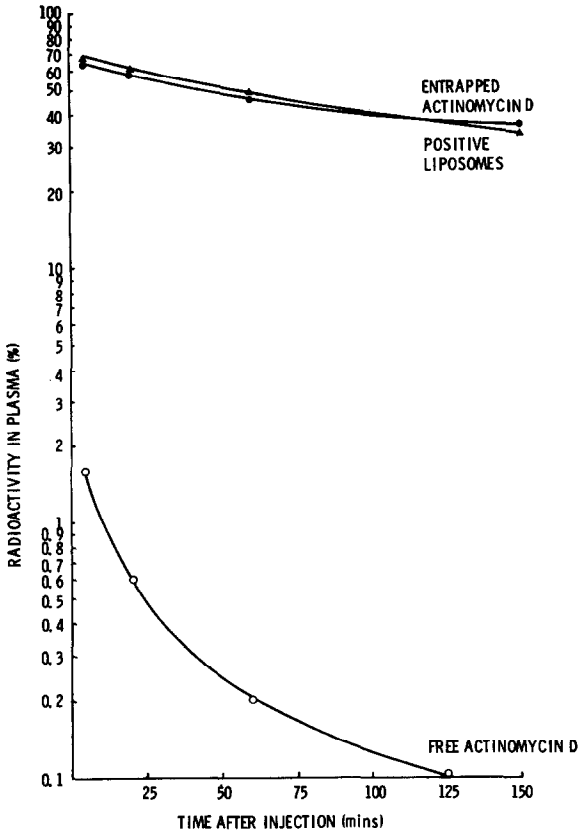

Fig. 3. Elimination from blood of non-entrapped and liposome-entrapped [^{3}H]actinomycin D. Rats were injected with 1.5 μg (2.1×10^{6} dpm) non-entrapped actinomycin D (○—○—○), 1.2 μg (5.5×10^{5} dpm) actinomycin D entrapped in the lipid phase of liposomes (●—●—●) or 0.21 mg (8.5×10^{4} cpm) liposome-entrapped [^{125}I]albumin (▲—▲—▲). Liposomes (2.5 mg lipid) were composed of egg lecithin, cholesterol and stearylamine (see legend to table 1). Each point is the average value from 2 to 3 rats.

Soon after injection of non-entrapped [^{14}C]penicillin most radioactivity was removed from the circulation (fig. 1) and some of it transiently recovered in the liver (22.7%), kidney (9.5%) and spleen (1% of the dose) (fig. 2). In contrast, not only entrapped [^{14}C]penicillin exhibited a slower rate of plasma disappearance (fig. 1) but half the radioactivity injected

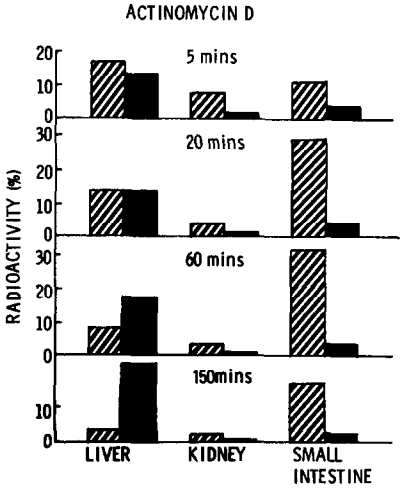

Fig. 4. Time course of uptake and persistence of [^{3}H]actinomycin D in rat tissues. Rats were injected with non-entrapped and liposome-entrapped actinomycin D as described in fig 3. Maximum uptake of radioactivity by the spleen was 1.2% and 4.7% for the non-entrapped and the entrapped drug respectively. Other details are given in the legend of fig. 2.

was found in the liver (42%) and spleen (8%, 20 min) and a considerable amount of it was still retained by these tissues 2.5 hr later (fig. 2). Injection of non-entrapped [^{3}H]actinomycin D also resulted in its rapid removal from the plasma (fig. 3). Radioactivity was partially recovered in the small intestine, liver and kidney (fig. 4), and although 2.5 hr later very little of it was left in the liver and kidney considerable amounts were held by the small intestine (18% of the dose). Entrapment of [^{3}H]actinomycin D in the lipid phase of liposomes led to a dramatic change in its rate of plasma removal to levels similar to those for entrapped albumin and 2.5 hr after injection 38% of the dose was still present in the circulation (fig. 3). Further, there was now little radioactivity uptake by the small intestine (5% at most) and kidney, there was more radioactivity in the spleen, and radioactivity uptake by the liver increased with time (23% of the dose in 2.5 hr, fig. 4 and legend).

Despite the rapid loss from the circulation of entrapped penicillin (presumably following its diffusion through the liposomal membranes) (fig. 1) it is apparent from tissue uptake values (fig. 2) that, as with actinomycin D entrapped in the lipid phase of liposomes, a considerable portion of this drug was directed by the carrier into the liver and spleen. However, as no attempt was made to identify tissue radioactivity, it is not known to what extent measured labels represent the original drugs.

Earlier work has shown that liposomes can transport enzymes in a latent state into the rat liver lysosomes [1] and the lysosomes of cultured macrophages and fibroblasts [11]. Once in lysosomes enzymes regain their activity towards substrates stored in lysosomes [11] presumbaly following the rupture of liposomal membranes by lysosomal lipases or otherwise. It is conceivable that in the present experiments administration of liposome-entrapped drugs has also led to a similar lysosomal localization of liposome-entrapped drugs in the liver and spleen. After disruption of the carrier's membranes drugs can then act either within the lysosomes or, following diffusion, in other cellular compartments (i.e. nucleus). Coupling of drugs to DNA [12] or other molecules [13] through bonds vulnerable to lysosomal enzymes has also been proposed as a possibility for chemotherapy through lysosomes [14]. The use of liposomes as drug(s) carriers requires disruption of the lipid bilayers by a lysosomal action which is common to the liberation of any entrapped drug. Owing to the chemical composition of liposomes and the insulation of the entrapped drugs from the environment (when diffusion is minimal) immunological reaction and development of drug resistance, due in some cases to changes in cell membrane permeability to drugs [15], might not occur. Further, it might be possible to direct liposomes to cells other than those of the liver and spleen through specific manipulations of the liposomal surface [1, 16]. Comparative studies on the biological effect of liposome-entrapped and non-entrapped drugs are now in progress.

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