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Effect of the Size of Liposomes on the Transfer and Uptake of Carboxyfluorescein by the Perfused Human Term Placenta

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Abstract

The effect of the size of liposomes on the uptake and transfer of the low molecular-weight, hydrophilic and polar molecule carboxyfluorescein has been determined across the perfused human term placenta.

Carboxyfluorescein-encapsulated neutral liposomes of three different sizes were prepared from equimolar concentrations of lecithin and cholesterol. Size distribution, encapsulation efficiency and stability of liposomes in blood-based media were determined. The concentration of carboxyfluorescein was measured spectrophotometrically. The transplacental transfer and placental uptake of free carboxyfluorescein (control data) were respectively $1{\cdot}9 \pm 0{\cdot}2$ and $5{\cdot}0 \pm 0{\cdot}7\%$ of initial dose. The placental uptake and foetal concentration of carboxyfluorescein were significantly increased by small liposomes ($P < 0{\cdot}05$), and reduced by large ($0{\cdot}82 \pm 0{\cdot}13\%$; $P < 0{\cdot}05$) and multilamellar liposomes ($0{\cdot}32 \pm 0{\cdot}11\%$). There was a negative correlation between liposome size and transplacental transfer ($y = -0{\cdot}53 + 0{\cdot}9x$; $r = 0{\cdot}96$; $P < 0{\cdot}001$; $n = 24$) and placental uptake of carboxyfluorescein ($y = -5{\cdot}9 + 6{\cdot}5x$; $r = 0{\cdot}84$; $P < 0{\cdot}001$; $n = 24$).

The study indicates that placental uptake and transfer rate of liposomal carboxyfluorescein were dependant upon the size of liposomes.

A drug-carrier system is used in clinical practice to increase the therapeutic efficacy of a drug with minimum side-effects. Of the various drug-delivery systems available, the therapeutic potential of the synthetic carrier liposomes has been investigated extensively (Poznansky & Juliano 1984; Lichtenberg & Barenholz 1988; Gregoriadis & Florence 1993). Liposomes are non-toxic, biodegradable, unilamellar or multilamellar concentric vesicles formed from naturally occurring phospholipids which have the ability to entrap and retain a wide range of drugs in either aqueous or lipid phases (Senior 1987). Liposomes can be classified according to their size (small unilamellar vesicles, multilamellar vesicles or large unilamellar vesicles), surface charge (neutral, negative or positive) and lipid composition (solid or liquid). Liposomally encapsulated therapeutic agents can be administered either orally or parenterally (Hwang 1987). Factors such as size, surface charge, lipid composition, dose, route of administration and retention of entrapped solutes in the circulation can influence the pharmacokinetics of liposomes.

In recent years liposomes have been used in clinical practice for treatment of metabolic disorders (Gregoriadis et al 1982), infectious diseases (Richardson 1983), systemic fungal infections (Ralph et al 1993), and to reduce the adverse effects of chemotherapeutic drugs (Gabizon et al 1994). The results of these clinical studies are encouraging and suggest that liposomal encapsulation of a drug can facilitate its selective organ delivery with a minimum of systemic side-effects (Senior 1987; Eichler et al 1988).

Despite the proven therapeutic value of liposomes in a number of clinical conditions, their role in preferential drug delivery during pregnancy has not been elucidated. We have recently shown that small unilamellar liposomes can increase the uptake and transfer of drugs across the perfused human term placenta (Bajoria & Contractor 1997). We hypothesize that large liposomes can prevent the transfer of drugs across the placenta, thereby minimizing foetal exposure. This assumption is based on the observation that the pharmacokinetics and tissue distribution of liposomes depend on their sizes (Leserman et al 1990). Furthermore, in-vitro and in-vivo studies indicate that large liposomes are internalized by Kupfer cells whereas small liposomes are taken up predominantly by the hepatocytes (Heath et al 1985). To test this hypothesis we undertook this study to determine the uptake and transfer of neutral liposomes of different sizes across the perfused human term placenta.

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Materials and Methods

Materials

Chromatographically pure egg phosphatidylcholine, and grade 1 dicetyl phosphate in 2:1 chloroform–methanol was purchased from Lipid Products (Nutfield, UK). Cholesterol and stearylamine were obtained from Sigma (Poole, UK). Sephadex
G-25 was obtained from Pharmacia, UK, carboxyfluorescein from Kodak, UK.

Preparation of liposomes

Liposomes of three different sizes were prepared from equimolar concentrations of phospholipid and cholesterol using standard techniques (Senior & Gregoriadis 1984). Briefly, a thin lipid film was prepared by rotary evaporation of lipid suspension in 2:1 chloroform–methanol under reduced pressure (400–700 mmHg) at 37°C for 45 min. The lipid film was hydrated by addition of carboxyfluorescein solution (250 mM; 5 mL) and left to swell for 2 h at room temperature (21°C). Multilamellar liposomes of uniform size were prepared by

sonicating the lipid suspension for 2 min at 4°C under N_2. For small liposomes sonication was performed for 30 min. The sonicated liposomal suspension was annealed for 2 h at room temperature and centrifuged (15 000 *g* for 30 min) to separate undispersed lipid aggregates and large multilamellar liposomes.

The large liposomes were prepared by the dehydration-rehydration method of Kirby & Gregoriadis (1984). The steps were essentially similar to those described for small and multilamellar liposomes with some modifications which included: the dry lipid film was hydrated with tris-saline buffer (2 mL) then sonicated for 30 min; the sonicated liposomal suspension was mixed with carboxyfluorescein (17 mM; 2 mL) and lyophilized overnight in a centrifugal freeze-drier at 0·1 torr (Speedivac Model 5PS, Edwards High Vacuum, UK); the lyophilized powder was re-hydrated with distilled water (100 μL; one-tenth of the total initial volume), then with phosphate-buffered saline (PBS) (0·9 mL) and allowed to anneal at room temperature for 1 h.

The free carboxyfluorescein was separated from encapsulated small liposomal carboxyfluorescein by gel filtration on a 45 × 1 cm Sephadex G-25 column equilibrated with tris buffer, pH 7·4. Liposomes were eluted as 3–4-mL fractions of orange-coloured suspensions immediately after the void volume. For multilamellar and large liposomes, encapsulated carboxyfluorescein was separated from free carboxyfluorescein by centrifugation of the liposomal suspension (130 mL) and tris-saline buffer (pH 7·4; 10 mM) at 75 000 *g* for 30 min at 4°C. The liposome pellet was re-suspended in tris-saline buffer and re-centrifuged at least two or three times to ensure complete removal of non-entrapped carboxyfluorescein. The purified liposomal pellet was resuspended in tris-saline buffer (2 mL) and stored at room temperature.

The phospholipid and cholesterol content of each liposomal preparation were measured. The percentage carboxyfluorescein encapsulated per nanomole of lipids was determined by measuring the latency of carboxyfluorescein using the equation $(F2 - F1)/F2 \times 100$, where F2 and F1 are total and free carboxyfluorescein, respectively.

Free carboxyfluorescein was measured by suspending the liposome (10 mL) in buffer, total carboxyfluorescein after disrupting the liposomal membrane with 1% Triton X-100 in PBS.

The efficiency of encapsulation of carboxyfluorescein was expressed as the percentage of initial dose added. The specific encapsulation efficiency was expressed as the percentage mg^{-1} of liposomal phospholipid. The size and the number of lamellae of the liposomes were determined by use of a Jeol 100 CX electron microscope at both low and high magnification after negative staining with 1% ammonium molybdate.

Assessment of permeability of carboxyfluorescein-containing liposomes in biological media

The stability (in terms of carboxyfluorescein retention) of liposomes was determined in diluted maternal and cord blood and compared with those for PBS. Heparinized maternal and cord blood (20 mL) were collected after the delivery. Liposomes (1 mL) were incubated in the media (5 mL) at 37°C in a water bath. Samples (0·1 mL) were taken in duplicate every 30 min for 4 h and diluted with PBS (3·9 mL). The samples were centrifuged (1500 g for 10 min) and the latency of carboxyfluorescein in the supernatant was determined.

Placental perfusion technique

The dual closed-circuit perfusion of isolated lobules of human term placenta was established with placentae obtained after vaginal or caesarean deliveries after 37 weeks of gestation. The perfusion of the isolated lobule was commenced within 10–15 min at 37°C under optimum physiological conditions of oxygenation, pressure, flow, osmotic pressure and pH (Bajoria & Contractor 1992). Closed-circuit perfusion of the foetal-placental circulations was established by cannulating the chorionic artery and vein with a perfusion pressure of 40–50 mmHg and a venous outflow of 6–9 mL min^{-1}. Maternal circulation was established with an arterial pressure of 15–18 mmHg and flow rate of 24–30 mL min^{-1} by placing five cannulae in the intervillous space. The maternal perfusate was prepared from autologous maternal blood obtained from the intervillous space and diluted with Tc-199 medium; it had a mean haematocrit value of 6 (range 4–9). The foetal perfusate comprised diluted autologous cord blood and had a mean haematocrit value of 14 (range 12–18). The maternal and foetal circulating volumes were 150–160 mL and 110–120 mL, respectively. Tissue oxygenation was maintained by oxygenating the maternal circulation with a mixture of 95% oxygen and 5% carbon dioxide. The diffusional viability of the perfused placenta was determined by measuring the rate of transplacental transfer of the freely diffusing marker creatinine. Only when the maternal-to-foetal transfer of creatinine at the end of 2 h fell within the pre-defined range of 8–16% of the initial dose (Eaton et al 1985) were the experiments considered valid. Experiments were abandoned if foetal perfusion pressure or venous outflow were not within physiological ranges or if the foetal circulating volume dropped by more than 2 mL as a result of hydrostatic fluid shift from the maternal circuit.

Experimental protocol

Just before the perfusion experiment, liposome-encapsulated carboxyfluorescein was separated from free carboxyfluorescein by chromatography on a Sephadex G-25 column. A single bolus dose of liposome-encapsulated carboxyfluorescein and creatinine (30 mg) was added to the maternal arterial cannulae distribution head over a period of 6 min (the time required for a single maternal circulation). Five experiments each were undertaken with 20 nM free carboxyfluorescein and with small and large liposomes. Six experiments were performed with multilamellar liposomes.

Foetal and maternal samples (2 mL) were taken and volumes were replaced with equal volumes of fresh perfusate. Maternal circulation was sampled at 15 min and then every 30 min, whereas foetal samples were taken at 15-min intervals for 2 h. Further maternal and foetal samples (0·5 mL) were taken for estimation of pH, pCO_2 and pO_2.

The intervillous space of the perfused placental lobule was washed with fresh perfusate (500 mL) to remove liposomal-encapsulated carboxyfluorescein from the intervillous space. The perfusate draining the intervillous space was collected in 20 mL samples. The perfused placenta was dissected from the non-perfused tissue and pressure-blotted to remove excess trapped perfusate; perfused placenta was then homogenized in PBS by means of an ultra-turrax high-speed homogenizer. A sample of the homogenate was centrifuged (3000 *g* for 15 min) and the carboxyfluorescein concentration measured in the supernatant.

At the end of the perfusion period, both circuits were drained and their volumes were measured. All samples were centrifuged (3000 *g* for 15 min) and the stability of the liposome was determined at each sample point by measuring carboxyfluorescein latency.

The concentrations of carboxyfluorescein in the maternal and foetal circulations and in the placenta were expressed as a percentage of the dose added after correction for the background activity, circuit volume, and the amount removed from the previous sample.

Chromatography

Maternal and foetal perfusates (2 mL) were applied to a sephadex G-25 column (45 × 2 cm) to separate liposome-encapsulated and free carboxyfluorescein. The column was pre-equilibrated with Tris buffer and eluted at room temperature with Tris-saline buffer. The elution rate was 0·63 mL min^{-1} and fractions of 1 mL each were collected. Each fraction was tested for carboxyfluorescein, phosphatidylcholine and cholesterol.

Analytical methods

The concentration of carboxyfluorescein was measured fluorimetrically at excitation and emission wavelengths of 490 and 520 nm, respectively, with a sensitivity of 1 nM mL^{-1} and a coefficient of variation of 4–7%.

The phospholipid content of the liposomes was assayed by colorimetry with a sensitivity of 5 μg mL^{-1} and a coefficient of variation of 8–10%.

The cholesterol content of the liposomes was assayed by colorimetry with a sensitivity of 5 μg mL^{-1} and a coefficient of variation of 5–10%.

The creatinine concentration was determined by colorimetric assay with a coefficient of variation of 7–12%.

Data analysis

All values were expressed as mean ± s.e.m. Student's *t*-test and two-way analysis of variance were used to compare data between groups. *P* values < 0·05 were considered as indicative of significance. Equilibrium between maternal and foetal circuits was determined when foetal/maternal ratios of the drug levels were close to unity. Integrated values of maternal (MAUC) and foetal (FAUC) concentrations of a drug (i.e. areas under the concentration–time curves) were calculated by use of the trapezoidal rule (Bajoria et al 1996).

Results

The small and large liposomes were unilamellar with mean diameter of 74 ± 0·9 and 147 ± 1·4 nm, respectively. The multilamellar liposomes had concentric lamellae and a mean diameter of 296 ± 1·7 nm (Table 1). When incubated in PBS the stabilities of the liposomes were comparable. However, when incubated in diluted maternal sera, the stability of small liposomes decreased steadily from 97·2 ± 2·3% at 60 min to 91·4 ± 3·9% at 4 h, and was significantly lower than that of large and multilamellar liposomes. The stability of large and multilamellar liposomes were similar (Table 2).

Maternal to foetal transfer of carboxyfluorescein constituted the control data (Fig. 1). The maternal concentration of carboxyfluorescein decreased steadily from 100% to 79·0 ± 1·5% at 120 min with a small linear increase in foetal levels to 1·9 ± 0·2% at 120 min with a foetal/maternal ratio of 0·022 ± 0·003 (Fig. 2). The MAUC and FAUC were 9251 ± 128% dose min^{-1} and 112 ± 12% dose min^{-1}, respectively. The placental uptake of carboxyfluorescein was 5·0 ± 0·7% with a placental clearance rate of 0·1 ± 0·006 $mLmin^{-1}$.

Small liposomes significantly increased the foetal concentration of carboxyfluorescein (3·7 ± 0·4%; $P < 0·001$), FAUC (186 ± 27% dose min^{-1}; $P < 0·01$) and foetal/maternal ratio (0·05 ± 0·005; $P < 0·01$). The maternal concentration (71·3 ± 1·2%; $P < 0·01$) and MAUC (8367 ± 83% dose min-1; $P < 0.001$) were significantly lower than the control data. The placental uptake of carboxyfluorescein entrapped in liposomes was higher than for the control group (15·2 ± 1·6%; $P < 0·001$). The stability of small liposomes in the maternal circulation was 98·5 ± 2·1% at 0 min and 93·2 ± 2·8% at 120 min.

In contrast, large liposomes significantly increased the maternal concentration (85·0 ± 1·7%; $P < 0·05$) and MAUC (9714 ± 110% dose min^{-1}; $P < 0·05$) and reduced the foetal concentration (0·8 ± 0·1; $P < 0·01$) and FAUC of carboxyfluorescein (43·5 ± 6·8% dose min^{-1}; $P < 0·001$) in comparison with the control data. Similarly, the foetal/maternal ratio (0·009 ± 0·002; $P < 0·01$) and placental uptake (3·0 ± 0·4%; $P < 0·05$) of carboxyfluorescein were less than the control data. The latency of carboxyfluorescein in the maternal circuit

Table 1. Characteristics of carboxyfluorescein-encapsulating liposomes of different sizes.

	Type of liposome		
	Small (n = 10)	Large (n = 10)	Multilamellar (n = 11)
Size (nm)	74 ± 0·9	147·0 ± 1·4	296 ± 1·7
Initial concentration of carboxyfluorescein (mM)	250	250	250
Initial concentration of phosphatidylcholine (μM)	66	66	66
Final concentration of phosphatidylcholine (μM)	58 ± 1·4	58 ± 1·0	55 ± 1·2
Initial concentration of cholesterol (μM)	66	66	66
Final concentration of cholesterol (μM)	57 ± 1·2	60 ± 0·7	56 ± 0·7
Percentage encapsulation	1·5 ± 0·2	24·4 ± 3·5*	2·1 ± 0·1
Latency of carboxyfluorescein	98·0 ± 0·3	98·6 ± 0·2	98·0 ± 0·3

All values are expressed as mean ± s.e.m. *$P < 0·01$ compared with results from small and multilamellar liposomes.

Table 2. Stability (%) of liposomes in biological media.

Type of liposome	Blood-based media				Phosphate-buffered saline	
	15 min	60 min	120 min	240 min	15 min	240 min
Small	98·2 ± 0·5	95·9 ± 0·4	92·7 ± 0·8	88·5 ± 0·7*	98·1 ± 0·6	97·3 ± 0·7
Large	99·0 ± 0·23	97·7 ± 0·3	96·9 ± 0·2	95·0 ± 0·4	98·7 ± 0·3	97·3 ± 0·5
Multilamellar	98·8 ± 0·42	98·2 ± 0·37	97·1 ± 0·6	95·5 ± 0·5	99·0 ± 0·2	98·0 ± 0·3

All values expressed as mean ± s.e.m. (n = 5). *$P < 0·05$ compared with large and multilamellar liposomes.

decreased from 97·2 ± 1·1% at 15 min to 95·6 ± 1·6% at 120 min.

The multilamellar liposomes significantly increased the maternal concentration (94·0 ± 0·9%; $P < 0·001$) and MAUC (10111 ± 47% dose min^{-1}; $P < 0·001$) of carboxyfluorescein compared with the control group. The foetal concentration (0·4 ± 0·2%; $P < 0·001$), FAUC (19·0 ± 7·0% dose min^{-1}; $P < 0·001$) of carboxyfluorescein and foetal/maternal ratio (0·003 ± 0·0005; $P < 0·01$) were markedly lower than the control data. The placental uptake of carboxyfluorescein from multilamellar liposomes was significantly lower than in the control group (1·3 ± 0·3%; $P < 0·001$). The latency of carboxyfluorescein in the maternal circuit showed minimum alteration from 97·4 ± 1·3% at 0 min to 94·2 ± 2·6% at 120 min.

Figs 1 and 2 compare the effect of liposomes of different sizes on the transfer of carboxyfluorescein across the placenta. The maternal concentration ($P < 0·001$), and MAUC ($P < 0·01$) of carboxyfluorescein were significantly higher with multilamellar liposomes than with large. Similarly, maternal concentration ($P < 0·001$), MAUC ($P < 0·001$) and foetal/maternal ratio ($P < 0·001$) for large liposomes were higher than for small. In the multilamellar liposome group the foetal concentration ($P < 0·01$), FAUC ($P < 0·01$), foetal/maternal ratio ($P < 0·01$) and placental uptake ($P < 0·01$) of carboxyfluorescein were significantly lower than for the large liposomes. The large liposomes resulted in significantly lower foetal concentration of carboxyfluorescein ($P < 0·001$) and FAUC ($P < 0·001$) and significantly lower foetal/maternal ratio ($P < 0·001$) and placental uptake ($P < 0·001$) than for the small liposome group.

Negative correlations were found between liposome size and placental transfer (y = 3·6 − 0·01x; r = 0·77; $P < 0·001$), placental uptake (y = 17·51 − 0·06x; r = 0·735; $P < 0·001$) and

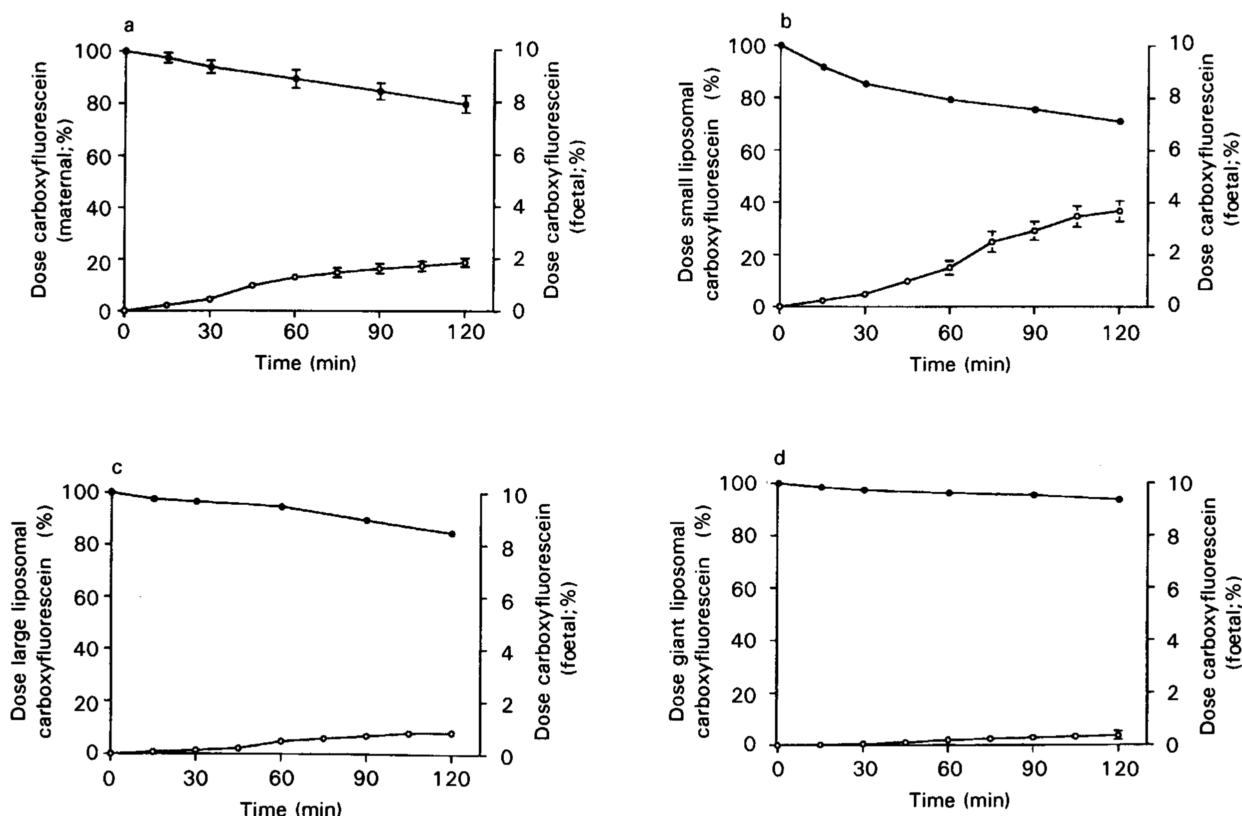

FIG. 1. Maternal–foetal transfer of (a) free carboxyfluorescein; (b) carboxyfluorescein encapsulated in small liposomes; (c) carboxyfluorescein encapsulated in large liposomes; and (d) carboxyfluorescein encapsulated in multilamellar liposomes: ●, maternal concentration; ○, foetal concentration. All data are expressed as a percentage of the initial dose of carboxyfluorescein added to the maternal circulation.

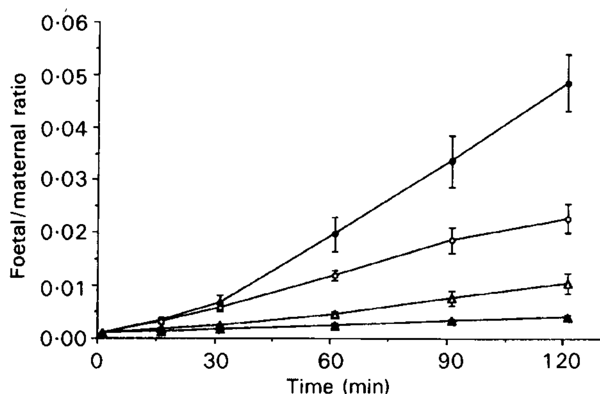

FIG. 2. Foetal-maternal ratio of carboxyfluorescein (○), and of small liposomes (●); large liposomes (△) and multilamellar liposomes (▲) containing encapsulated carboxyfluorescein. All values are expressed as mean ± s.e.m.

FAUC ($y = 216 - 0{\cdot}73x$; $P < 0{\cdot}001$) of carboxyfluorescein (Fig. 3).

Chromatography data

The chromatogram of the maternal perfusate showed two distinct peaks, the first occurring in fractions 4 to 8 and the second in fractions 17 to 23 (Fig. 4a). The first peak was that of liposome-encapsulated carboxyfluorescein as is evident from the presence of liposomal phospholipid and results from determination of the latency. The second peak was that of free carboxyfluorescein, and coincided with the peak in the chromatogram of free carboxyfluorescein (Fig. 4a). The chromatogram of the foetal perfusate contained only one peak which corresponded to that of free carboxyfluorescein (Fig. 4b). No intact liposomes were detectable in the foetal circulation. Similar results were obtained with large (Figs 4c and 4d) and multilamellar liposomes (Figs 4e and 4f).

Discussion

This study shows that transplacental transfer of a carboxyfluorescein can be modulated by varying the size of neutral liposomes. Small liposomes significantly enhanced placental uptake and passage of carboxyfluorescein whereas large and multilamellar liposomes prevented its transfer. We used carboxyfluorescein as a model substance to study the transfer kinetics of liposomes in the maternal–placental foetal unit. Although the use of membrane-bound liposomal markers such as [^{14}C]cholesterol has a number of advantages (Szoka et al 1979; Hwang 1987), its major limitation is that tissue uptake and clearance might not be similar to those of the liposomes. In contrast, clearance of the aqueous phase marker carboxyfluorescein was found to be similar to that of liposomes because it is an inert, hydrophilic, polar molecule which does not bind to plasma proteins or lipids (Szoka et al 1979; Damen et al 1981; Hwang 1987). Furthermore, as picomolar con-

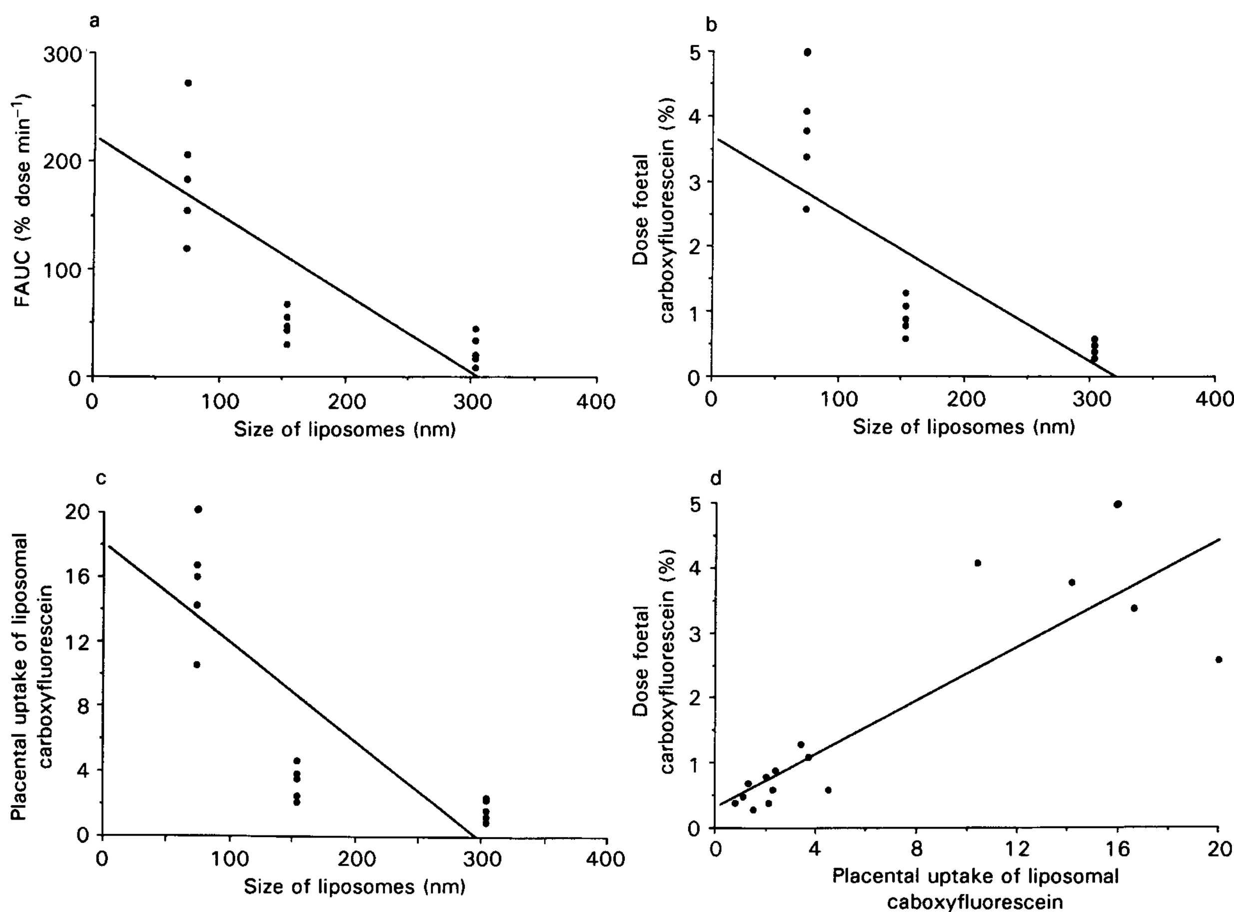

FIG. 3. Relationship between size of the liposomes and (a) foetal AUC, (b) transplacental transfer, and (c) placental uptake of carboxyfluorescein; (d) shows the relationship between placental uptake and foetal concentration of carboxyfluorescein.

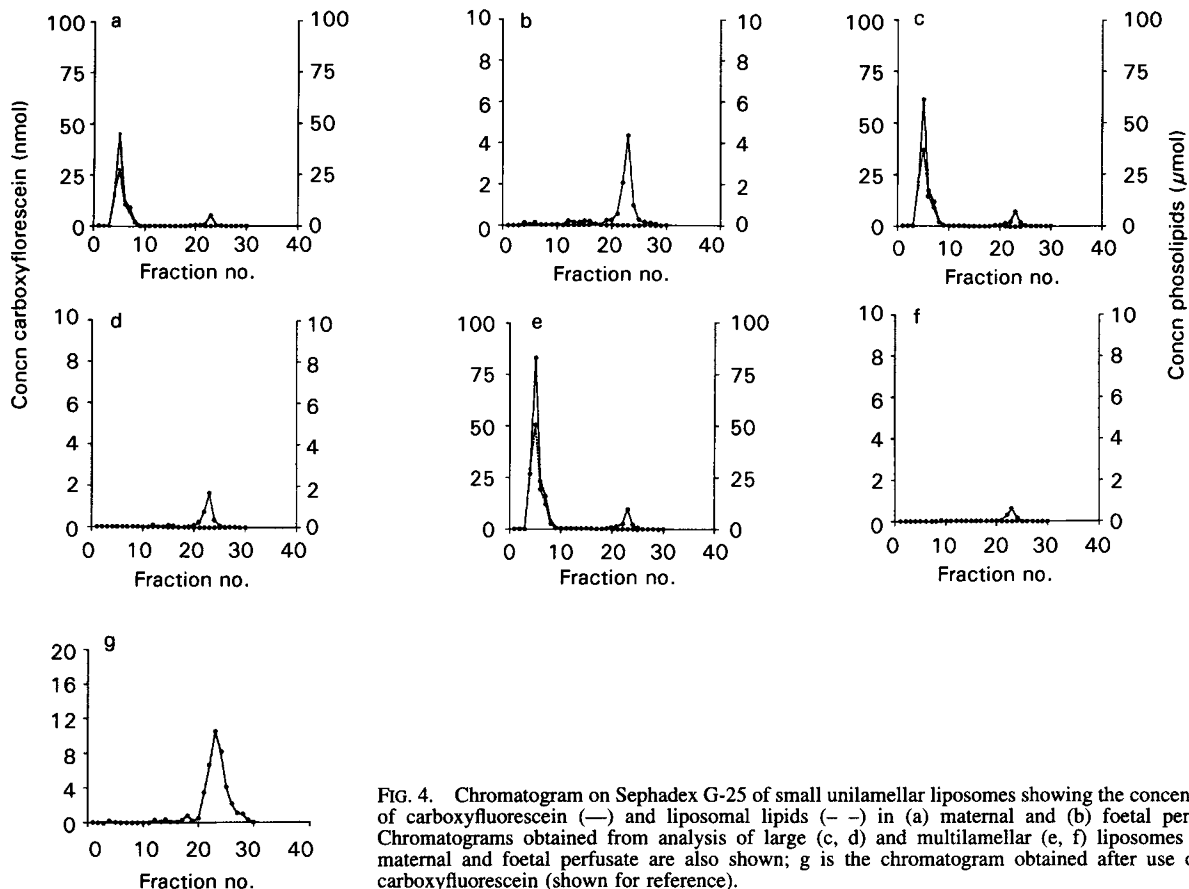

FIG. 4. Chromatogram on Sephadex G-25 of small unilamellar liposomes showing the concentration of carboxyfluorescein (—) and liposomal lipids (– –) in (a) maternal and (b) foetal perfusate. Chromatograms obtained from analysis of large (c, d) and multilamellar (e, f) liposomes in the maternal and foetal perfusate are also shown; g is the chromatogram obtained after use of free carboxyfluorescein (shown for reference).

centrations of carboxyfluorescein can be quantified fluorimetrically, it has been used extensively to study the pharmacokinetics and tissue distribution of liposomes (Kirby & Gregoriadis 1984; Senior 1987).

We studied liposome transfer by use of an in-vitro model of the perfused human term placenta. Our data show that carboxyfluorescein crosses the placenta sparingly. This was attributed to its high polarity and hydrophilicity and not to its molecular size because drugs with similar molecular weights cross the placenta freely (Reynolds & Knott 1989). Similarly, many investigators have shown that the rate of transfer of polar molecules such as chlorazepate and cimetidine depend upon their hydrophilicity rather than on their molecular weight, and occurs through the hypothetical water-filled pores of the membrane (Illsley et al 1985; Ching et al 1987; Bajoria & Contractor 1992).

Our data show that the rate of transfer of small liposomes was significantly higher than of large or multilamellar liposomes. The reason for this remains unclear. It is unlikely to be a result of experimental error because we have characterized this model extensively (Contractor et al 1984; Bajoria & Contractor 1992). Furthermore, to minimize intra-experimental error we used transplacental transfer of a freely diffusing, inert molecule creatinine as an internal marker (Eaton et al 1985). As creatinine transfer was comparable in all the experiments, it seems unlikely that the differential transfer rate and uptake were because of failure to establish juxtaposition between maternal and foetal circulation.

Another reason for increased uptake and transfer of carboxyfluorescein by small liposomes could be the transfer of intact liposomes across the placenta. Failure to detect any intact liposomes or liposomal lipids in the foetal perfusates make this proposition unlikely.

It is possible that increased transplacental transfer of carboxyfluorescein by small liposomes could be a consequence of leakage in the maternal circulation. The dependence of liposome instability on size has been shown to be a result of greater affinity of the irregular surface of small liposomes to high-density lipoprotein or to apolipoprotein present in the serum (Scherphof et al 1983; Jonas 1984). Furthermore, use of cholesterol has been shown to stabilize the liposomal membranes in a blood-based medium (Senior & Gregoriadis 1982). In this study, despite the use of an equimolar ratio of cholesterol to phospholipid, we failed to make small liposomes with stability similar to that of large and multilamellar liposomes. Although use of washed autologous maternal and foetal blood cells suspended in the tissue-culture media would have made the liposome stable without compromising the oxygenation of the perfused placenta (Contractor et al 1984; Bajoria et al 1996), we used autologous blood as maternal and foetal perfusate. This experimental design was largely based on the evidence that tissue uptake of liposome depends upon adsorption of serum proteins and opsonin to the liposomal membrane (Senior & Gregoriadis 1982). Given the evidence that the concentration of free carboxyfluorescein in the experiments with small liposomes was 100-fold less than in the control

group, it seems unlikely that increased uptake and transfer of carboxyfluorescein by small liposomes is a result of their increased leakage in the maternal circulation.

It seems, therefore, more likely that the increased transfer of carboxyfluorescein by small liposomes was a result of increased uptake by the placenta. This assumption is based on the observation that placental uptake of free carboxyfluorescein was five times higher than in the small liposome group. Taken together this suggests either that carboxyfluorescein is metabolized during its transport from the maternal to foetal circulation or is retained within the trophoblast cells. Given the evidence that carboxyfluorescein is an inert molecule and does not undergo biotransformation, accumulation within the trophoblast cells rather than metabolism seems the more likely explanation. The absence of intact liposomes in the placental homogenate further indicates that liposomes are degraded after internalization and that the carboxyfluorescein thus released is largely retained within the placental tissue.

Although this study was not designed to elucidate the mechanism by which liposomes are taken up by the placenta, on the basis of literature evidence and our observations some deductions can be made. It has been shown that cellular uptake of liposomes occurs either by endocytosis or by fusion (Papahadjopoulos et al 1973; Hwang et al 1987; Straubinger et al 1990). On the basis of the finding of this study that transfer of carboxyfluorescein from maternal to foetal circulation depends upon the size of liposomes, we propose that placental uptake of liposomes occurs by endocytosis. However, transfer of carboxyfluorescein could still occur by liposomal size-dependent fusion because the extent of fusion could depend upon the number of bilayers in the liposomes used. This possibility cannot be eliminated because the small liposomes used in this study were unilamellar whereas large liposomes had more than one bilayer. Further studies are warranted to elucidate the mechanism by which liposomes are internalized by the trophoblast cells.

In conclusion, our data suggest that uptake and transplacental transfer of liposomally encapsulated substances depend upon liposome size. If similar results can be obtained using liposome-encapsulated commonly used therapeutic agents, liposomes might find application as a non-invasive model for selective drug delivery to the mother or the foetus.

References

Bajoria, R., Contractor, S. F. (1992) Transfer of heparin across the human perfused placental lobule. J. Pharm. Pharmacol. 44: 952–959

Bajoria, R., Contractor, S. F. (1997) Transfer and uptake of small unilamellar liposomes of different surface charge. Pediatr. Res., in press

Bajoria, R., Oteng Natim, E., Fisk, N. M. (1996) Transfer and metabolism of thyrotropin-releasing hormone across the perfused human term placenta. J. Clin. Endocrinol. Metab. 81: 3476–3482

Ching, M. S., Mihaly, G. W., Morgan, D. J., Date, N. M., Hardy, K. J., Smallwood, R. A. (1987) Low clearance of cimetidine across the human placenta. J. Pharmacol. Exp. Ther. 241: 1006–1009

Contractor, S. F., Eaton, B. M., Firth, J. A., Bauman, K. F. (1984) A comparison of the effects of different perfusion regimes on the structure of the isolated human placental lobule. Cell Tissue Res. 237: 609–617

Damen, J., Regts, J., Scherphof, G. (1981) Transfer and exchange of phospholipid between small unilamellar liposomes and rat plasma high density lipoproteins. Dependence on cholesterol content and phospholipid composition. Biochim. Biophys. Acta. 665: 538–545

Eaton, B. M., Browne, M. J., Contractor, S. F. (1985) Maternal to foetal movement of creatinine as a measure of perfusion efficiency and diffusional transfer in the isolated human placental lobule. Placenta 6: 341–346

Eichler, H. G., Senior, J., Stadler, A., Gasic, S., Pfundner, P., Gregoriadis, G. (1988) Kinetics and disposition of fluorescein-labelled liposomes in healthy human subjects. Eur. J. Clin. Pharmacol. 34: 475–479

Gabizon, A., Izacson, R., Libson, E., Kaufman, B., Uziely, B., Catane, R., Ben-Dor, C. G., Rabello, E., Cass, Y., Peretz, T. (1994) Clinical studies of liposome-encapsulated doxorubicin. Acta Oncol. 33: 779–786

Gregoriadis, G., Florence, A. T. (1993) Recent advances in drug targeting. Trends Biotechnol. 11: 440–442

Gregoriadis, G., Weereratne, H., Blair, H., Bull, G. M. (1982) Liposomes in Gaucher type I disease: use in enzyme therapy and the creation of an animal model. Prog. Clin. Biol. Res. 95: 681–701

Heath, T., Lopez, N. G., Papahadjopoulos, D. (1985) The effects of liposome size and surface charge on liposome-mediated delivery of methotrexate-gamma-aspartate to cells in vitro. Biochim. Biophys. Acta 820: 74–84

Hwang, C. (1987) Liposome pharmacokinetics. In: Ostro, M. (ed.) Liposomes: from Biophysics to Therapeutics. Marcel Dekker, New York, pp 109–156

Hwang, K. J., Padki, M. M., Chow, D. D., Essien, H. E., Lai, J. Y., Beaumier, P. L. (1987) Uptake of small liposomes by non-reticuloendothelial tissues. Biochim. Biophys. Acta 901: 88–96

Illsley, N. P., Hall, S., Penfold, P., Stacey, T. E. (1985) Diffusional permeability of the human placenta. Contrib. Gynecol. Obstet. 13: 92–97

Jonas, A. (1984) A review of plasma apolipoprotein A: interactions with phosphatidylcholines. Exp. Lung Res. 6: 255–270

Kirby, C., Gregoriadis, G. (1984) Dehydration-rehydration vesicles: a simple method for high-yield drug entrapment in liposomes. Biotechnology 12: 979–984

Leserman, L., Machy, P., Leonetti, J. P. (1990) Targeted liposomes and intracellular delivery of macromolecules. Horiz. Mem. Biotechnol. 95–102

Lichtenberg, D., Barenholz, Y. (1988). Liposomes: preparation, characterization and preservation. Methods Biochem. Anal. 33: 337–462

Papahadjopoulos, D., Poste, G., Schaeffer, B. (1973) Fusion of mammalian cells by unilamellar lipid vesicles: influence of lipid surface charge, fluidity and cholesterol. Biochim. Biophys. Acta 323: 23–42

Poznansky, M. J., Juliano, R. L. (1984) Biological approaches to the controlled delivery of drugs: a critical review. Pharmacol. Rev. 36: 278–325

Ralph, E. D., Barber, K. R., Grant, C. W. (1993) Clinical experience with multilamellar liposomal amphotericin B in patients with proven and suspected fungal infections. Scand. J. Infect. Dis. 25: 487–496

Reynolds, F., Knott, C. (1989) Pharmacokinetics in pregnancy and placental drug transfer. Oxf. Rev. Reprod. Biol. 11: 389–449

Richardson, V. J. (1983) Liposomes in antimicrobial chemotherapy. J. Antimicrob. Chemother. 12: 532–534

Scherphof, G., Van-Leeuwen, B., Wilschut, J., Damen, J. (1983) Exchange of phosphatidylcholine between small unilamellar liposomes and human plasma high-density lipoprotein involves exclusively the phospholipid in the outer monolayer of the liposomal membrane. Biochim. Biophys. Acta 723: 595–599

Senior, J. (1987) Fate and behaviour of liposomes in vivo: a review of controlling factors. Crit. Rev. Ther. Drug Carrier Syst. 3: 123–193

Senior, J., Gregoriadis, G. (1982) Stability of small unilamellar liposomes in serum and clearance from the circulation: the effect of the phospholipid and cholesterol components. Life. Sci. 30: 2123–2136

Senior, J., Gregoriadis, G. (1984). Methodology in assessing liposomal stability in the presence of blood, clearance from the circulation of injected animals and uptake by tissues. In: Gregoriadis, G. (ed.) Liposome Technology. CRC Press, Boca Raton, Florida, pp 264–282

Straubinger, R. M., Papahadjopoulos, D., Hong, K. L. (1990) Endocytosis and intracellular fate of liposomes using pyranine as a probe. Biochemistry 29: 4929–4939

Szoka, F. C., Jacobson, K., Papahadjopoulos, D. (1979) The use of aqueous space markers to determine the mechanism of interaction between phospholipid vesicles and cells. Biochim. Biophys. Acta 551: 295–299