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The effect of stabilising agents on the organ distribution of lipid emulsions

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Summary

The effect of different emulsifying agents on the organ deposition and blood clearance of i.v. administered fat emulsions has been investigated in rabbits using gamma scintigraphy. Emulsions stabilized by egg lecithin were sequestered by the liver to a limited extent. Such liver uptake was enhanced by coemulsification using gelatin, thereby exploiting a receptor-mediated process involving fibronectin. Liver uptake was almost totally eliminated if the block copolymer, poloxamine 908, was employed as the emulsifier. The blood clearance and liver uptake of emulsions were little affected by the incorporation of model drugs, lasalocid and amphotericin B.

Introduction

Colloidal carrier systems used for site-specific drug delivery can comprise not only solid microspheres made from materials such as starch, albumin, gelatin or synthetic polymers but also lipid microspheres in the form of fat emulsions and liposomes (Davis et al., 1984; Gregoriadis, 1988; Juliano, 1987; Davis et al., 1985). The different types of emulsion systems and their applications have been reviewed in detail by Davis et al. (1985).

The fate of any colloidal particle (including emulsion droplets) administered i.v. will depend upon physicochemical characteristics such as particle size, surface charge and surface hydrophobicity (Davis and Illum, 1986, 1988). For some applications, it is intended that the emulsion system should be rapidly sequestered by cells at specific sites, for example phagocytic cells in the liver or at sites of inflammation. For other applications, particularly those involving blood replacement, a long circulation lifetime will be desired.

Emulsions intended for parenteral nutrition are formulated using vegetable oils as the disperse phase and phospholipid as the emulsifier with the objective that they are not recognised as "foreign" by the body. They can be considered as being artificial chylomicrons and enter the fat metabolism pathway through the adsorption of apolipoproteins (largely CII and CIII) and the subse-

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quent action of lipoprotein lipase. However, small differences in the composition of the phospholipid emulsifier can render such systems recognised as foreign in the body and then they are removed rapidly by the various elements of the reticuloendothelial system (Davis et al., 1987a). It has been shown that this clearance process can be enhanced deliberately if emulsions are coated with materials that will encourage the adsorption of specific blood components. For example, emulsions coated with gelatin are rapidly removed from the circulation through the specific uptake of the blood component fibronectin. This glycoprotein is able to trigger receptors on phagocytic Kupffer cells residing in the sinusoids of the liver (Gudewicz et al., 1980).

Previous studies by ourselves and others (Davis et al., 1987b; Kimura et al., 1986; Illum et al., 1982, 1986), have demonstrated conclusively that the particle size and especially the surface nature of a colloidal carrier can dictate the subsequent uptake of these materials by macrophages. For example, Davis and Hansrani (1985) showed that emulsions stabilised using phospholipids of various compositions and non-ionic surfactants in the form of block copolymers of polyoxyethylene and polyoxypropylene, were phagocytosed to different extents by mouse peritoneal macrophages. There was no clear correlation between uptake and surface charge, suggesting that surface polarity was a more important factor. The same type of block copolymer has been studied in vivo using emulsion systems by Jeppsson and Rossner (1975) and Geyer (1967). Emulsion droplets were retained within the systemic circulation of rabbits for long periods of time and the effect was related to the molecular weight of the stabilising agent.

Previously we have demonstrated that it is possible to direct model particles in the form of polystyrene microspheres after i.v. injection in the rabbit to different sites (Illum and Davis, 1987; Illum et al., 1987). Large polystyrene particles (greater than 10 μm) were rapidly removed by the capillary beds of the lungs by a process of mechanical filtration. Smaller polystyrene particles were removed by the Kupffer cells of the liver (Illum et al., 1982), but, by coating them with polymer materials such as poloxamine 908 (a polyoxyethylene-polyoxypropylene-ethylene diamine block copolymer), it was possible to keep such particles in circulation for extended time periods (Illum et al., 1987). Furthermore, particles smaller than approximately 100 nm in size coated with poloxamer 407 (a polyoxyethylene-polyoxypropylene block copolymer), were neither sequestered by the liver, nor did they remain in the general circulation. Instead, they were deposited in the bone marrow (Illum and Davis, 1987). The mechanisms that may determine such selective distribution of colloidal particles are presently being investigated.

The present investigation was conducted to ascertain whether a biodegradable emulsion system, produced using a vegetable oil and stabilised with the poloxamine 908, would demonstrate the same characteristics as found for the non-biodegradable polystyrene particles. We describe here studies conducted in the rabbit where the blood and organ level distribution of labelled fat emulsions have been evaluated. Additional studies have also been undertaken using the emulsifiers routinely employed for i.v. fat emulsions and associated drug delivery systems, namely egg lecithin and hydrogenated soy lecithin. The mediation of emulsion uptake in the liver through the use of gelatin as an additional coating agent has also been evaluated.

Emulsion systems are now finding increasing use for drug delivery. Therefore, the influence of the incorporation of two model drug agents, amphotericin B and lasalocid, on the in vivo distribution of the emulsion systems has been investigated. Amphotericin is known to be intercalated into the surface layer of phospholipid-stabilized emulsions (Davis et al., 1987a); Washington et al., 1988) while lasalocid is a lipid soluble chelating agent that can dissolve in the oil phase (Shastri et al., 1987) with a small proportion at the oil-water interface (Aranda and Gomez-Fernandez, 1986).

Materials and Methods

Materials

Egg lecithin (Lipoid E80) was provided by Geistlich and Son, Chester, U.K. The hydro-

genated soy lecithin was obtained from Ajinomoto Chemicals, Japan. Lasalocid sodium, amphotericin B, soybean oil and gelatin were all from Sigma Chemical Co., U.K. The indium-111 chloride used for labelling the lasalocid was obtained from Amersham International, U.K. and the ^{123}I-sodium iodine used for labelling the oil from Harwell Laboratories, U.K. The poloxamine 908 was provided by BASF Chemical Company, Cheadle, U.K. All other chemicals were of reagent grade.

Preparation of emulsions.

The soybean oil was labelled with iodine-123 using a modification of the method described by Lubran and Pearson (1958). In this method the iodine is covalently attached across double bonds of unsaturated components in the oil. The labelling efficiency was found to be 20–25% (West, 1988). The labelled oil was mixed with non-labelled oil to obtain an appropriate level of activity and emulsified using an ultrasonic probe (Dawe Soniprobe) with either 1.2% w/v egg lecithin, 1.2% w/v hydrogenated soy lecithin, 2% w/v poloxamine 908, as stabilisers. The surfactant was dissolved in the aqueous component of the emulsion and then mixed with the oil and sonicated at medium intensity for 10 min. The compositions of the emulsion systems are shown in Table 1. The volume of the final oil phase was 10% in all experiments and the emulsion droplet size about 250 nm as determined by photon correlation spectroscopy (PCS) (Malvern Instruments, Malvern, U.K.). The particle size obtained was very similar to that for the commercial fat emulsion product Intralipid 10% (Kabi Vitrum, Sweden).

In one experiment, the egg lecithin-stabilised system was mixed with 0.3% w/v gelatin as described by Tonaki et al. (1976). The egg lecithin (1.2% w/v) was dispersed in the soybean oil by use of a sonic bath; 0.3% w/v aqueous solution of gelatin was added and the mixture was sonicated for 10 min.

Preparation of emulsion containing lasalocid

In order to be able to follow concurrently the fate of the emulsion and an entrapped drug, lasalocid labelled with a different gamma emitter to that in the oil, ^{111}In was incorporated into the emulsion system stabilised with poloxamine 908 in one set of experiments. The concentration of lasalocid was 0.1% w/v.

The labelling of the lasalocid was performed by a method based on that used by Hwang et al. (1982). 68 μl of $^{111}InCl_3$ (in 0.04 N hydrochloric acid) was added to 500 μl of lasalocid sodium in ethanol (0.01 mg/ml) and incubated at room temperature for 15 min. The solvents were evaporated with nitrogen. Hydrochloric acid of pH 2.5 was

TABLE 1

Composition of emulsion systems

Emulsion system	Oil phase	Stabiliser	Added drug
(1)	soybean (10%)	egg lecithin (1.2%)	–
(2)	soybean (10%)	hydrogenated soy lecithin (1.2%)	–
(3)	soybean (10%)	poloxamine 908 (2.0%)	–
(4)	soybean (10%)	egg lecithin (1.2%) gelatin (0.3%)	–
(5)	soybean (10%)	egg lecithin (1.2%)	lasalocid (1 mg/ml)
(6)	soybean (10%)	egg lecithin (1.2%)	amphotericin B (0.5 mg/ml)
(7)	soybean (10%)	poloxamine 908 (2.0%)	lasalocid (1 mg/ml)
(8)	soybean (10%)	poloxamine 908 (2.0%)	amphotericin B (0.5 mg/ml)

used to wash the residue. One hundred μl of ethanol was added and the solvents were again evaporated. Soybean oil (0.15 g) was added to the tube and the lasalocid solution complex was dissolved by means of a sonic bath treatment. The labelling efficiency was found to be about 50%.

Preparation of emulsion containing amphotericin B

The emulsion system containing amphotericin B and stabilised by poloxamine 908 was prepared by dissolving the amphotericin B in methanol. The stabiliser dissolved in water was then added. The methanol was removed thoroughly leaving a crude solubilised preparation. The oil phase was then added and the system emulsified using a sonic probe. The final concentration of the amphotericin B was 0.5 mg/ml. The other materials had the same concentrations as before.

Animal experiments

Female New Zealand White rabbits of about 2.5 kg were divided into groups of 3 and injected via the marginal ear vein with about 1 ml of the appropriate emulsion system (Table 1). The amounts of activity used were 5–10 MBq for the ^{123}I labelling and about 1.3 MBq for the ^{111}In labelling.

The distribution of the emulsion systems in the animals was followed using gamma scintigraphy (GEC Maxicamera). Dynamic images were recorded for 15 min (20 frames) after the injection and static images (1 min duration) were recorded for up to 6 h at suitable time intervals and stored on a computer for analysis. Regions of interest were created around the liver/spleen area and the activity level in this site was calculated and corrected for radiodecay and background activity. In the dual isotope study corrections were made for "scatter-down" of indium-111 activity (245 keV) into the lower photopeak of iodine-123 (159 keV).

In order to follow the level of activity in the circulation, blood samples (1 ml) were withdrawn from the vein in the contralateral ear at suitable time intervals and the activity measured after dilution, using a conventional gamma counter (Ortec). Those samples containing both 123-iodine and 111-indium were counted twice, the day after collection of the blood samples and 4–5 days after to allow for the 123-iodine counts to become negligible relative to the 111-indium counts.

Results

The emulsifying agents used for the stabilisation of the emulsion systems were found to have a significant effect on the recorded levels of activity for the labelled emulsion in the liver/spleen area (Table 2). Thus, for the emulsion stabilised with egg lecithin the liver/spleen activity was found to be in the order of 41% while that for the emulsion stabilised with poloxamine 908 was found to be approximately 27% of the administered dose (Fig. 1A). When the data were corrected for the observed blood level activities, i.e. 25% of the circulating activity the corresponding liver/spleen uptake (Triplett et al., 1985) values were 34% and 7%, respectively (Fig. 1B).

The corresponding blood level activity data for the emulsion systems stabilised with egg lecithin and poloxamine 908 show that the former system is cleared much more quickly from the bloodstream than the latter emulsion system with half-lives of clearance being 5 min and 208 min, respectively, as calculated using a first order kinetics analysis (Fig. 2). The data have been corrected for the presence of small quantities of free 123-iodine originally present in the emulsion, which will be cleared very rapidly from the circulation and gives

TABLE 2

The percentage activity in the liver/spleen of emulsion systems stabilised with various stabilisers (data not corrected for blood pool) (mean ± S.E.M.)

Emulsion system	Percentage activity after		
	15 min	1 h	4 h
1	39.0 (±2.0)	36.0 (±3.0)	34.0 (±2.0)
2	33.0 (±1.0)	31.0 (±1.0)	32.0 (±1.0)
3	25.0 (±0.1)	28.0 (±2.0)	27.0 (±2.0)
4	58.0 (±1.0)	39.0 (±2.0)	34.0 (±1.0)
5	40.7 (±2.0)	–	–
6	41.0 (±2.0)	31.0 (±1.0)	30.0 (±5.0)
7	32.0 (±1.0)	32.0 (±1.0)	31.0 (±2.0)
8	27.0 (±1.0)	26.0 (±1.0)	24.0 (±1.0)

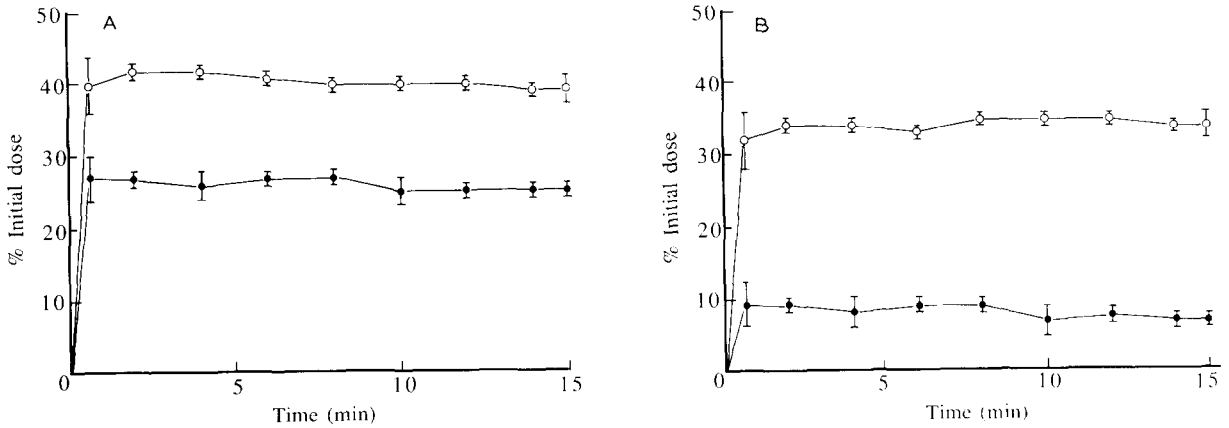

Fig. 1. The uptake of an i.v. administered fat emulsion labelled with iodine-123 in the liver/spleen region of the rabbit as measured by gamma scintigraphy (mean ± SEM) ($n = 3$). A: uncorrected data. B: data corrected for blood-pool activity in the liver. ○, 1.2% Egg lecithin; ●, 2% poloxamine 908.

an apparent initial rapid fall in blood level for both systems.

When the egg lecithin-stabilised emulsion system was mixed with 0.3% w/v gelatin it was found that the amount of activity recorded in the liver/spleen area increased from about 41% to about 60% of the administered activity (Fig. 3). The emulsion stabilised by hydrogenated soy lecithin showed a liver/spleen uptake very similar to the one obtained for the emulsion stabilised with egg lecithin (Fig. 3).

The incorporation of the labelled lasalocid into both the emulsion systems stabilised by egg lecithin

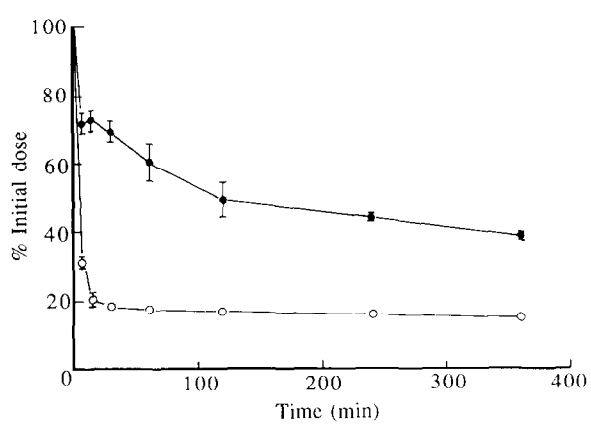

Fig. 2. The blood clearance of an i.v. administered fat emulsion labelled with iodine-123 in the rabbit (mean ± S.E.M.) ($n = 3$). ○, 1.2% Egg lecithin; ●, 2% poloxamine 908.

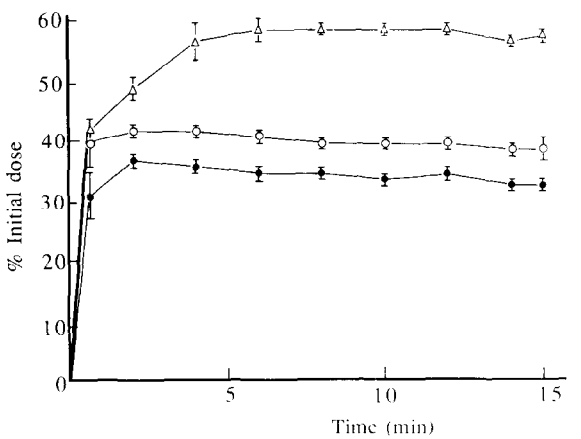

Fig. 3. The uptake of an i.v. administered fat emulsion labelled with iodine-123 in the liver/spleen region of the rabbit as measured by gamma scintigraphy (mean ± S.E.M.) ($n = 3$). ○, 1.2% Egg lecithin; △, 1.2% egg lecithin plus 0.3% gelatin; ●, 1.2% hydrogenated soy lecithin.

TABLE 3

The percentage activity in the liver/spleen of fat emulsion stabilised by poloxamine 908 and containing lasalocid as expressed by the activity of the 123-iodine labelled oil and the 111-indium labelled lasalocid (mean ± S.E.M.)

	Percentage activity after		
	15 min	1 h	4 h
Soybean oil iodine-123	32.0 (±1..0)	32.0 (±1.0)	31.0 (±2.0)
Lasalocid indium-111	36.0 (±1.0)	34.0 (±1.0)	34.0 (±1.0)

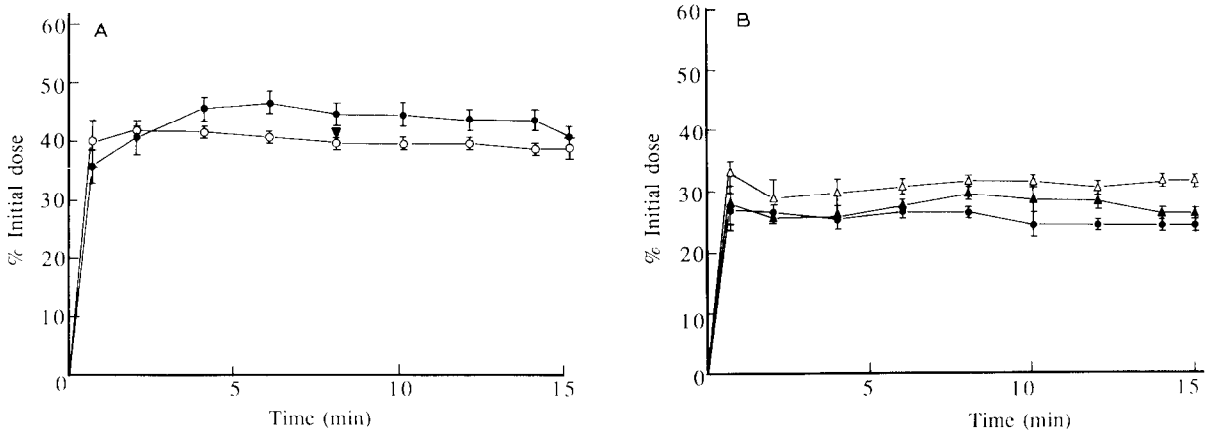

Fig. 4. The uptake of an i.v. administered fat emulsion labelled with iodine-123 in the liver/spleen region of the rabbit as measured by gamma scintigraphy (mean ± S.E.M.) ($n = 3$). A: egg lecithin as emulsifier. B: poloxamine as emulsifier. ○, No added drug; ●, 0.5 mg/ml amphotericin B; ▼, 1.0 mg/ml lasalocid (one time point only in A).

and by poloxamine 908 gave rise to a slight increase in liver/spleen deposition (Fig. 4). Similarly, the incorporation of amphotericin B into these two emulsion systems resulted in an increase of liver/spleen activity from 41% to about 45% and from 25% to about 30%, respectively (Fig. 4).

The corresponding blood level curves for the two emulsion systems with incorporated lasalocid or amphotericin B are shown in Fig. 5. Apparently, the incorporation of lasalocid or amphotericin B only influenced the rate of clearance of the emulsions stabilised with poloxamine 908 whereas the rates of clearance for the egg lecithin stabilised emulsions are not significantly different from each other.

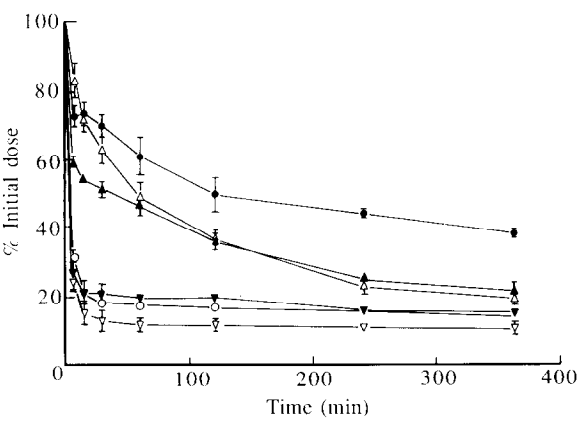

Fig. 5. The blood clearance of an i.v. administered fat emulsion labelled with iodine-123, in the rabbit (mean ± S.E.M.) ($n = 3$). ○, Egg lecithin emulsifier, no drug; ●, poloxamine emulsifier, no drug; ▽, egg lecithin emulsifier, 1 mg/ml lasalocid; ▼, egg lecithin emulsifier, 0.5 mg/ml amphotericin B; △, poloxamine emulsifier, 1 mg/ml lasalocid; ▲, poloxamine emulsifier, 0.5 mg/ml amphotericin B.

By comparing the liver/spleen activities of 123-iodine from the labelled oil and ^{111}indium-from the labelled lasalocid in the poloxamine 908-stabilised emulsion in Table 3 it is apparent that the levels are very similar for the 4 h duration of the experiment. Thus, it is indicated that the lasalocid was retained within the emulsion during the time course of the experiment.

Discussion

By using the block-copolymer poloxamine 908 as the emulsifying agent it has been possible to decrease considerably the deposition of a fat emulsion system in the liver and spleen as determined by gamma scintigraphy. A measured liver sequestration as low as 7% is found, as compared to 35% for an emulsion stabilised by egg lecithin (Fig. 1). The reduced liver uptake for the poloxamine 908 stabilised emulsion corresponded well with the blood clearance data that demonstrated a delayed clearance from the circulation. These blood clearance data are in agreement with those

presented by Jeppsson and Rossner (1975) for fat emulsions stabilised by egg lecithin and poloxamers 188 and 338. These authors found that the elimination rates of emulsions stabilised with various poloxamers were much lower than the rate found for the emulsion stabilised with egg lecithin and that the rate was lowest for the higher molecular weight surface active agent (poloxamer 338). Similarly, Davis and Hansrani (1982) studied the effect of the emulsifier on the fate and deposition of fat emulsions and found that the nature of the emulsifying agent affected clearance parameters as measured by gamma scintigraphy, block copolymers having the greatest effect.

Fat emulsions stabilized by phospholipids are used widely in parenteral nutrition and are intended to be 'mimics' of the natural fat particles, the chylomicrons that originate from dietary fat. A properly formulated fat emulsion should undergo the same clearance mechanism as endogenous chylomicrons, namely lipolysis at the endothelial surface of capillaries in adipose tissue and skeletal and cardiac muscles. This process is mediated by the uptake of the apoproteins CII and CIII and the action of lipoprotein lipase (Lenzo et al., 1988). The liver has no involvement in the clearance at nascent chylomicrons but has an important part to play when the "remnants" from the hydrolysis process are transported to the liver and removed by a receptor mediated process (under the control of a different apoprotein). Whether or not commercial fat emulsion are taken up to a significant extent by the macrophages in the liver and spleen has long been a matter of debate (Allen and Murray, 1986). We have found previously that Intralipid caused a small degree of blockade of the reticuloendothelial system (as tested by the administration of a test dose of labelled tracer colloid) (Davis et al., 1987b). Other fat emulsions are known to cause greater blockade and one system, based upon soybean oil and emulsified with soy lecithin, has even been proposed as tests for reticuloendothelial function (Scholler, 1973). The extent to which a chylomicron copy will be removed by the liver is related to a recognition process involving the competitive adsorption of opsonizing blood components such as albumin, gamma globulins, complement, fibronectin, fibrinogen, in addition to the already mentioned apoproteins. It is appreciated that small differences in phospholipid composition of the emulsifier (for example soy vs egg lecithin) can effect this recognition process. In the liposome field there is a number of reports dealing with this aspect and how the judicious choice of phospholipid mixture can dictate blood clearance and liver uptake (Allen et al., 1984).

At first sight, it is surprising that the emulsion prepared using a hydrogenated phospholipid had a similar clearance and liver uptake behaviour to that prepared with an unhydrogenated egg lecithin, since the physical state of the emulsifier layer is to be expected to have a significant effect on its fate in the body (Ziak et al., 1984; Lenzo et al., 1988; Clark and Derksen, 1987). It is believed that the phospholipid emulsifying layer must be in a fluid state for the correct insertion of apoproteins (Morrisett et al., 1977). Emulsions with "solid" phospholipid emulsifying layers are not hydrolysed by lipoprotein lipase and therefore have different blood clearance and liver uptake characteristics to those that are so hydrolysed. Measurement of the phase transition temperature of the hydrogenated soy lecithin used in the present work by DSC (Perkin Elmer) gave a value of 34.0 °C. Consequently, the phospholipid layer would be in a fluid state at body temperature. Moreover, it is probable that adsorbed serum components can reduce the phase transition temperature of phospholipids in vivo (Jain and Zakim, 1987).

The addition of gelatin to an emulsion leads to the expected increase in liver uptake. It has been reported that gelatinized colloids take up the opsonic factor fibronectin from the blood and subsequently these particles are cleared more rapidly and to a greater extent by the Kupffer cells of the liver through a receptor-mediated process (Molnar et al., 1987; Gudewicz et al., 1980).

The uptake of opsonizing blood components onto the surface (emulsion) particles can be minimized by giving them a hydrophilic surface (Davis and Illum, 1986, 1988). This effect has already been well demonstrated for solid polystyrene microspheres (Illum et al., 1987). The present results, obtained with the block copolymer poloxamine

908 used as an emulsifier, confirm the same effect as for biodegradable triglyceride particles.

The incorporation of model 'drugs' into emulsions has only a small effect on blood clearance and liver uptake. Lasalocid, which is incorporated both in the oil phase and at the interface, has less effect than amphotericin B which is intercalated only at the interface. However, for both materials the observed effects are small. Thus, the uptake of an emulsion (and by implication any drug retained therein) can be directed into or away from the liver depending upon the choice of the emulsifying agent and clinical need.

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