Long-Circulating Emulsions (Oil-in-Water) as Carriers for Lipophilic Drugs

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Purpose. Rapid clearance of parenterally administered oil-in-water emulsions from blood by the reticuloendothelial system (RES), mainly macrophages of the liver and spleen, has been one of the major obstacles for delivering lipophilic drugs to cells other than those in the RES. The purpose of this study therefore is to overcome this problem and develop emulsions that will have prolonged blood circulation time. ***Methods.*** A series of amphipathic polyethyleneglycol (PEG) derivatives have been included as co-emulsifier into emulsions composed of Castor oil and phosphatidylcholine. The effect of amphipathic PEG on reducing the RES uptake and prolonging the blood circulation of the emulsion particles has been tested in vivo using mice as an animal model. ***Results.*** Inclusion of PEG derivatives such as Tween-80 or dioleoyl N-(monomethoxypolyethyleneglycol succinyl)phosphotidylethanolamine (PEG-PE) into emulsions composed of Castor oil and phosphatidylcholine decreases the RES uptake and increases blood residence time of the emulsions. The activity of PEG derivatives in prolonging the circulation time of emulsions depends on the PEG chain length (PEG2000≥PEG5000>PEG1000, Tween-80) and the PEG density on emulsion surface. ***Conclusions.*** Inclusion of amphipathic PEG as emulsifier into oil-in-water emulsions is a very effective method to prolong the blood half life of the emulsions. Emulsions with long circulating half life in blood should be very useful as a delivery vehicle for lipophilic drugs.

KEY WORDS: long-circulating emulsions; polyethyleneglycol; reticuloendothelial system.

INTRODUCTION

Perhaps the most important problem of conventional chemotherapy is the lack of specificity of a drug for its target. Side effects arise from the administration of the large doses needed to achieve a reasonable concentration of the drug at the target site. Because of these problems, the development of drug delivery systems that can mediate the site-specific delivery of drugs and concentrate the drug at the target site with minimal effects on surrounding normal tissues has been pursued.

Among the different approaches to drug delivery (1), liposomes have probably attracted the most attention in the last 20 years due to their potential to encapsulate high concentrations of hydrophilic drug molecules in their aqueous compartment. There are many successful examples of using liposomes as a carrier to deliver hydrophilic drugs into cells (2, 3). For example, it has been demonstrated that anticancer drugs (4-6), toxins (7), enzymes (8), oligonucleotides (9) and genes (10, 11) can be successfully delivered to the target cells and express their biological activity. However, applications of liposomes in delivering hydrophobic compounds are limited. This is because the amount and types of lipophilic drugs that are able to be incorporated into the liposome bilayer are rather low. Small amounts of hydrophobic drugs incorporated into the liposome bilayer will likely change the properties of the liposome and result in the loss of drug delivery activity. Therefore, a carrier that can encapsulate and deliver hydrophobic drugs has been long sought by the researchers.

A lipid emulsions stabilized with emulsifiers such as phospholipids are an attractive candidate for lipophilic drug delivery. Oil-in-water (o/w) emulsions are composed of two major parts, the oil core and emulsifiers on the surface. Such emulsions have a similar structure to that of liposomes at the outer surface. The major differences between o/w emulsions and liposomes are that emulsions have one layer of amphipathic molecules such as phosphatidylcholine on the surface and the inside core is filled with oil. Liposomes, on the other hand, contain an outer bilayer of amphipathic molecules such as phospholipids with a large aqueous compartment(s) inside. The great potential of using emulsions as drug carrier for lipophilic molecules includes use as carriers for sustained release and for targeted drug delivery. Lipid emulsion systems have many appealing properties as drug carriers: for example, they are biodegradable, biocompatable and physically stable. Unlike liposomes, lipid emulsion can be prepared on a large industrial scale and are relatively stable below 25°C for long periods (12). Most importantly, a considerable amount of lipophilic drugs can be solubilized in the hydrophobic core of the emulsion particles.

The progress of emulsion drug delivery for parenteral use has been very slow. Among the many serious obstacles that can account for such slow development, the most important is emulsion uptake by the reticuloendothelial system (RES). When emulsions are administered intravenously they are rapidly taken up by the mononuclear phagocytes in the liver and spleen (13, 14). This may be advantageous for delivery of drugs to the RES, but it is certainly problematic for delivery to targets outside the RES. Because of this problem, the potential of emulsions as drug delivery systems for parenteral use have not been fully explored. In this report, we demonstrate that amphipathic polyethylene glycols can be used to decrease the uptake of emulsions to the RES and prolong their circulation time in blood. These results suggest that amphipathic PEG, especially PEG derivatives of phospholipids may be useful emulsifiers for the development of emulsions as delivery vehicles for lipophilic drugs.

MATERIALS AND METHODS

Materials

Castor oil and carboxylflourescein were purchased from Sigma. Tween 80 was from Fisher Scientific. Phosphatidylcholine (PC) from egg yolk was from Avanti Polar Lipids. $^{111}InCl_3$ (carrier-free) was from New England Nuclear. Diethylenetriaminepentaacetic acid stearylamide (DTPA-SA) and dioleoyl N-(monomethoxy polyethyleneglycol succinyl) phosphatidylethanolamine (PEG-PE) were kindly provided

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by Dr. Leaf Huang (Department of Pharmacology, University of Pittsburgh). Synthesis of these compounds have been described in previous reports (15, 16). Animals were purchased from Sprague and Dawley Inc.

Preparation of Emulsions

Castor oil (1.5 mg) diluted in chloroform was mixed with PC and PEG derivatives (0.6 mg of total surfactants) at different weight ratios. A trace amount of hexadecyl ^{3}H-cholestanyl ether (^{3}H-CE, as oil marker) and ^{111}In-DTPA-SA (as lipid marker) were also included. The organic solvents were evaporated under a stream of nitrogen gas. The lipid film was then vacuum desiccated at 4°C overnight to remove residual organic solvents. One ml of phosphate buffered saline (PBS, pH7.4) was then added and the mixture was allowed to hydrate for 1 hour at room temperature. Lipid suspension was then mixed with a vortexer and subsequently homogenized for 3-4 min using a tissue tearer at a speed of about 20,000 rpm. The average diameter of the resulting emulsion particles was determined by quasi elastic laser light scattering with a Coulter N4SD particle analyzer.

Stability of Emulsions

Emulsion stability was determined by monitoring the size changes in the emulsions as the function of time using laser light scattering measurements. Stability of emulsions in serum was determined in the presence of 80% mouse serum at 37°C. One hundred μl of emulsions (0.21 mg total lipids) were incubated with 400 μl of freshly collected mouse serum at 37°C for 2 hours. The mixture was then loaded on a column (Biogel A1.5, 1.5 × 30 cm) and eluted from the column using PBS (pH 7.4) as the elution buffer. Aliquot from each fraction of the elution was counted for both ^{111}In and ^{3}H by gamma and scintillation counting respectively. The elution profile for each marker was then constructed. To check whether serum causes the size change of emulsion, the particle size of the serum treated emulsions was analyzed after chromatographic removal of the serum components. The average diameter of the emulsion particles before and after serum treatment was determined by light scattering.

Proportion of Liposomes in Emulsion Preparations

The proportion of liposomes in the emulsion preparations was determined by the method of Lundberg (17) using carboxylfluorescein (CF) as water soluble marker. After evaperation of the organic solvent and vaccuum desication of the lipid mixture, the dried lipid film was hydrated in a PBS buffer containing 50 mM CF. As reference, the same amount of phosphatidylcholine (PC only) as used in emulsion preparation was hydrated and homogenized under the identical conditions. Under such conditions (PC as only lipid conponent), liposomes are formed and CF is entrapped inside the liposomes. In all the preparations, equal amount of ^{3}H-CE was included as the lipid marker. The liposome (PC only) and emulsion preperations were then loaded separately on a column (Biogel A1.5) to separate free CF from that entrapped inside the liposomes. Aliquats of both liposomes (PC only preparations) and emulsions (both containing the same amount ^{3}H counts) were analized in the presence of detergent (Triton X-100, 0.2 %) for the fluorescence intensity in a fluorospectrophotometer at $\lambda ex = 490$ nm, $\lambda em = 520$ nm. The fluorescence intensity of each emulsion preparations was compared with the fluorescence intensity of the pure liposome preparation (PC only). The ratio (presented as percentage) of the CF intensity by a given amount of emulsions (as measured by the ^{3}H counts) to that of the pure liposomes (contaitining same amount ^{3}H counts) was presented as percentage of liposome contamination in emulsion preparations.

Biodistribution Studies

Biodistribution studies were performed in mice (male, NIH-C3, 20-25g) as described previously (18). Emulsions (0.21 mg total lipids in 100 μl) labeled with ^{111}In-DTPA-SA were injected via tail vein. At various time intervals, animals were sacrificed and ^{111}In-radioactivity in different organs was analyzed using a Beckman gamma-counter. The percent injected dose in the blood was determined by assuming that the total volume of blood is 7.3% of the body weight (19). Correction for blood contamination into other organs was made using correction factors obtained using ^{51}Cr-labeled red blood cells (18).

RESULTS AND DISCUSSION

We employed Castor oil and PC as the basic composition for our studies. This composition has been widely used as a model system for emulsion studies. The effect of polyethylene glycol based surfactants including Tween 80, lipid derivatives of PEG with different chain length on liposome contamination and average diameter of emulsion particles is shown in Table I. The weight ratio of oil phase to surfactant was kept at 1.5 to 0.6. As shown in Table I, a large amount of CF was found in emulsion preparations when PC was used as the only emulsifier. Approximately 20% liposome contamination was found in such formulations. Substitution of PC by increasing amounts of PEG-PE 2000 decreases the percentage of liposome contamination. Less than 5% of liposome contamination was obtained when 66% of PC was substituted with PEG-PE 2000. Also, at this ratio, substitution of PC with either Tween 80 or PEG-PE with PEG chain length of 1000 or 5000 is as efficient as PEG-PE 2000 in decreasing liposome contamination.

It has been shown in liposome biodistribution studies that particle size is one of the most important parameters affecting their circulation time in vivo (18, 20). We have previously shown that liposomes composed of phosphatidylcholine and cholesterol mixed with about 6.3% monosiaganglioside exhibit a long blood half life. These liposomes have diameters ranging from 80 to 300 nm. However, liposomes with the same composition showed very short resident times in blood when their diameters were either below or above this range. Liposomes with a diameter of less than 80 nm were rapidly removed by the liver while those with diameters greater than 300 nm were filtered by the spleen macrophages (20). As is seen in Table I, the average diameter of the particles in our emulsions was around 200 nm. This is in the range of particle size where their in vivo circulation properties are dependent only on their surface properties.

To check stability, emulsions labeled with both ^{111}In-DTPA as a marker for the emulsifier and ^{3}H-CE for the oil phase were incubated at 37°C for two hours either in freshly

Table I. Effect of Amphipathic PEG Derivatives on Particle Size and Proportion of Liposomes in Emulsion Preparations Composed of Castor Oil and Phosphatidylcholine[a]

Composition	Weight ratio (mg)	Liposome contamination (%)	Average diameter[b] (nm)
Oil/PC[c]	1.5:0.6	19.6	235 ± 16
Oil/PC/PEG-PE 2000	1.5:0.55:0.05	17.2	196 ± 6
	1.5:0.5:0.1	11.5	189 ± 16
	1.5:0.4:0.2	7.2	177 ± 7
	1.5:0.2:0.4	2.8	184 ± 25
Oil/PC/Tween-80	1.5:0.2:0.4	3.7	203 ± 10
Oil/PC/PEG-PE 1000	1.5:0.2:0.4	3.9	205 ± 8
Oil/PC/PEG-PE 5000	1.5:0.2:0.4	1.8	182 ± 8

[a] Each preparation (1 ml) contained 1.5 mg of oil and 0.6 mg surfactants. The analysis of liposome contamination in these preparations is described in Materials and Methods sections.
[b] The average diameter of emulsions was obtained from the unimodel analysis from light scattering measurements.
[c] Emulsions were prepared at 50°C.

collected mouse serum (80%) or PBS (pH7.4). The mixture was then passed through a column (Biogel A1.5, 1.5 × 30 cm). Each elution fraction was then counted for both ^{111}In and ^{3}H and the ratio of the isotopes in these fractions was compared to that of the emulsions without serum treatment. Peak fractions for ^{111}In and ^{3}H were pooled and the particle size was analyzed. No separation of the two markers nor change in particle size under our experimental conditions were observed (data not shown). In fact, the particle size of the preparation composed of oil/PC/PEG-PE 2000 (1.5:0.2:0.4, weight ratio) did not change after a period of 12 weeks at either room temperature or 4°C, suggesting good physical stability of this formulation (Figure 1).

For in vivo biodistribution studies, the emulsions were labeled with ^{111}In tracer bound to a lipophilic derivative of DTPA chelator that has been widely used for biodistribution studies (16, 18, 20). The weight ratio for the emulsions was kept at 1.5:0.2:0.4 (Oil/PC/X, where X represents different PEG derivatives). Figure 2 shows the effect of different PEG derivatives on the biodistribution of these emulsions. Within 30 min after intravenous injection, these emulsions distributed mainly into two organs, the blood and liver. Less than 5% of the injected emulsions regardless of composition were found in the other organs. The activity of PEG derivatives in prolonging emulsion circulation time is dependent on the chain length of the PEG. PEG-PE 2000 and PEG-PE 5000 showed a similar activity in keeping the emulsions in the blood. Thirty min after emulsion administration, there were still about 60-70% injected dose circulating in blood. PEG-PE 1000 and Tween 80 exhibited similar activity with 47% injected dose remaining in blood. High concentrations in blood were accompanied by a low accumulation in liver (Figure 2).

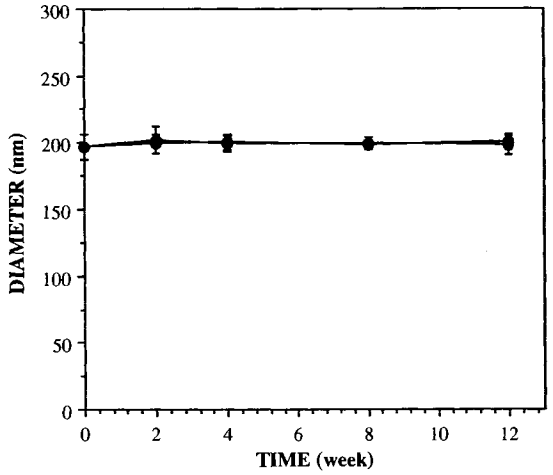

Fig. 1. Stability of emulsions. The emulsions composed of castor oil (1.5mg) phosphatidylcholine(0.2mg) and PEG-PE 2000 (0.4 mg) were prepared in 1 ml of PBS (pH 7.4) as described in the Methods. The change of the particle size under different storage conditions was analyzed using particle size analyzer. (●) room temperature, (○) 4°C.

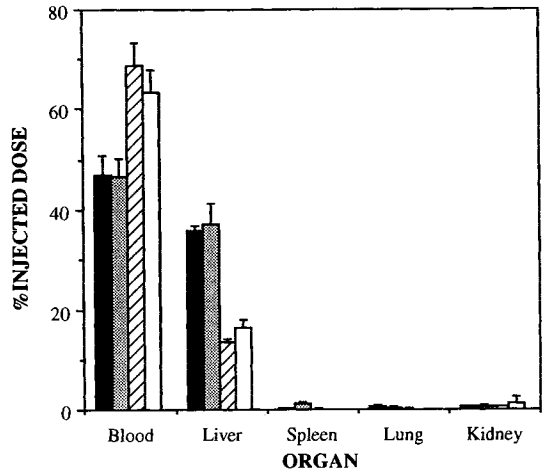

Fig. 2. Effect of amphipathic PEG derivatives on the tissue distribution in mice (n = 3) of emulsions. The ratio by weight of the lipids in the emulsions was kept constant (oil/PC/PEG derivatives = 1.5:0.2:0.4). Emulsions contain Tween-80 (■), PEG-PE 1000 (▤), PEG-PE 2000 (▨) and PEG-PE 5000 (□). The distribution was analyzed 30 min after intravenous injection.

Emulsions of three different compositions were prepared for kinetic studies in vivo: oil/PC (1.5/0.6, weight ratio), oil/PC/Tween-80 (1.5:0.2:0.4, weight ratio) and oil/PC/PEG-PE 2000 (1.5:0.2:0.4, weight ratio). Time dependent distribution of the emulsions in mice after intravenous injection was examined (Figure 3). Emulsions composed of oil and PC were rapidly removed from blood. By 6 hour after injection, these emulsions had been almost completely cleared from the blood by the liver. Different activities of Tween 80 and PEG-PE-2000 in prolonging the circulation time of emulsions were also evident. An approximate 5 fold increase in blood concentration of emulsions containing PEG-PE 2000 was observed at 6 hour while only 2 fold increase was found for emulsions containing Tween-80. Thus, all amphipathic PEG derivatives significantly enhanced the residence time of the emulsions, although the activity of PEG-PE 2000 seemd to be better than the others (Figures 2 and 3).

The effect of increasing PEG-PE 2000 concentration on prolonging the resident time of the emulsions are shown in Figure 4. Increasing the PEG-PE 2000 to PC ratio, while the ratio of oil to surfactants was kept constant, enhanced the residence time of emulsions. This increase was accompanied by a decrease in liver uptake. For example, at a ratio of 0.5 (PEG-PE 2000:PC = 0.2:0.4, weight ratio), about 65% of the injected emulsion was found in blood and less than 20% in the liver. Further increasing this ratio, however, resulted in a plateau in blood concentration of the emulsions, suggesting that the concentration effect of PEG-PE in prolonging emulsion circulation time had reached the saturation concentration.

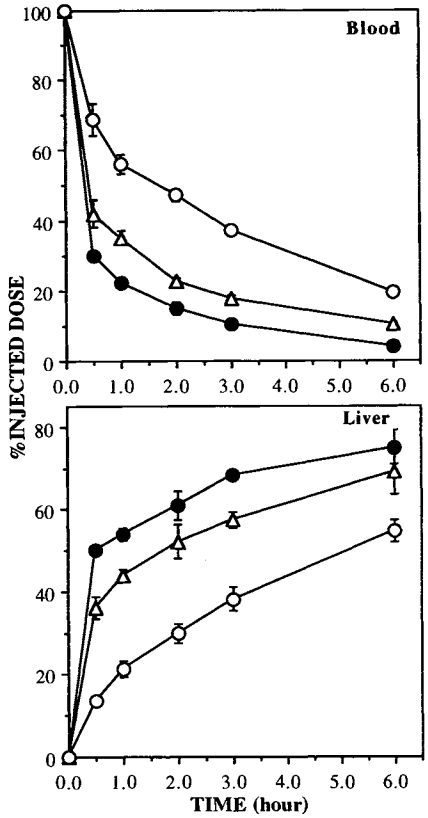

Fig. 3. Time-dependent distribution of emulsions in blood and liver of experimental mice (n = 3). (●) oil/PC (1.5:0.6, weight ratio), (△) oil/PC/Tween-80 (1.5:0.2:0.4, weight ratio), (○) oil/PC/PEG-PE 2000 (1.5:0.2:0.4, weight ratio).

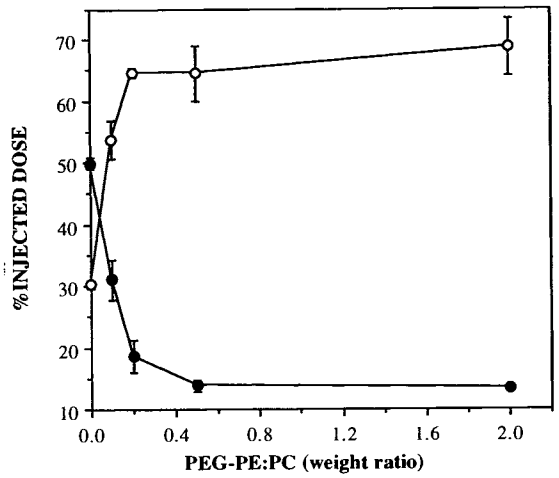

Fig. 4. Effect of increasing ratio of PEG-PE 2000 to PC on emulsion distribution in blood (○) and liver (●) of experimental mice (n = 3). The oil to surfactants (PC + PEG-PE 2000) ratio was kept at 1.5:0.6 (weight ratio). The emulsion distribution was analyzed 30 min after intravenous injection.

PEG has been widely used as a modifier to increase the blood circulation time of particulate drug carriers. For example, inclusion of PEG-PE into liposomes has been shown to significantly enhance the circulation time of liposomes (16, 21, 22), resulting in a higher tumor accumulation (23). PEG-PE has also been used to prolong the circulation of polymeric nanospheres (24). It has recently been shown that conjugation of PEG to the surface of nanoparticles reduced their uptake by the RES and increased blood residence time (25). The results described in this report demonstrate that this same concept can also been used to prolong the circulation time of oil in water emulsions. Coating of the emulsion surface with PEG may increase the hydrophilicity of the surface such that nonspecific interaction with liver Kupffer cells is reduced. Alternatively, PEG may sterically prevent the binding of serum opsonins to the emulsion surface thereby greatly reducing the affinity for the RES (26). Similar results demonstrating the steric stabilization effect of PEG-PE on emulsions have also recently reported (27).

Our results have important implications in studing the clinical use of a great number of hydrophobic drugs available. A major obstacle currently hampering the use of these drugs is their lipophilicity. The surfactants that are commonly used to formulate these drugs into oil in water emulsions preferentially directs the emulsions particles to the RES, causing a short blood circulation time and contributing to liver toxicity. The results presented in this report demonstrate that it is now possible to use PEG-PE as surfactant to sustain the blood concentration of these drugs such that more drug is available to cells other than those in the RES. Furthermore, the availability of long-circulating emulsions should make it possible to design emulsions capable of delivering hydrophobic drugs specifically to a target tissue.

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