Biochimica et Biophysica Acta, 1029 (1990) 91–97
Elsevier

BBAMEM 75011

Liposomes for the sustained drug release in vivo

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(Received 13 April 1990)

Key words: Model membrane; Drug carrier; Lipid vesicle; Cell uptake; Biodistribution

New lipidic carriers suitable for the sustained drug release in vivo are presented. They consist of middle sized, compact phospholipid vesicles with one or up to few lipid bilayers which are sterically stabilized with a small amount of large-head phospholipids. As an example, phosphatidylcholine (PC) liposomes casted with up to 10 mol% of phosphatidylethanolamine with a covalently attached polyethyleneglycol 5000 headgroup (PE-PEG) are discussed. Such vesicles exhibit a very long circulation time after an i.v. administration in mice; the improvement over pure phosphatidylcholine liposomes within the first 24 h exceeds 8000%, at this point nearly 25% of the applied PE-PEG liposomes being still in the circulation. This advantage is a consequence of reduced phagocytosis of the lipidic carriers, as shown by an in vitro assay with blood monocyte cells in the flow cytometric experiments. For example, after 6 h incubation with THP-1 monocyte cells in human plasma the difference between the uptake of standard distearoylphosphatidylcholine (DSPC) and novel liposomes containing 10% distearoylphosphatidylethanolamine-PEG is by 1000%. Vesicles with 2.5 mol% DSPE-PEG are also taken-up via phagocytosis relatively slowly. But the latter vesicles, moreover, retain most of the enclosed model-drug carboxyfluorescein after an incubation in plasma. The rate of permeation of the encapsulated substance from such DSPE-PEG liposomes is below 2.4% per h. This is by approximately a factor of two less than for pure DSPC liposomes; vesicles with a higher PE-PEG content are inferior in this respect. Long circulation time and high retention of the newly developed liposomes open up ways for the future systemic use as such stabilized drug carriers for the therapeutic applications in vivo.

Introduction

Lipid vesicles (liposomes) are believed to have appreciable potential as drug carriers, especially in the therapy of cancer and infections [1–6]. They can incorporate essentially any type of drug, either in the polar vesicle interior or in the hydrophobic regions of the lipid bilayers. But along with this general virtue they also possess a big disadvantage common to all particulate carriers: after systemic application, standard liposomes are rapidly removed from the blood, mainly by the cells of the mononuclear phagocyte (MPS, or in the old terminology, RHS) system. Phagocytosized lipid vesicles typically end-up in liver and spleen [7–9].

Procedures were developed for increasing the life-time of microspheres in blood. Polystyrene particles coated with poloxamer 908, for example, are significantly less attractive for the phagocytic cells in vitro. Therefore, they also persist longer in blood and are better suited for carrying drugs in vivo [10].

Over the last years, attempts have been made to create also long-lived liposomes for the sustained drug release along the same lines. This was mainly tried by inclusion of various negatively charged bilayer components (for example, glycolipids, phosphatidylinositol [11] and monosialoganglioside [12–14]), or by the surface-modification of the carrier liposomes with sialoglycopeptides [15]. In several cases, notable success was achieved, especially when modifications involved lipids apt to form rigid bilayers; vesicles containing distearoylphosphatidylcholine, cholesterol, and sphingomyelin are some examples. Less successful were attempts to ensure comparable benefit by covering the lipid vesicles with a layer of fully bio-compatible, bio-

Abbreviations: DSPC, 1,2-distearoyl-*sn*-glycero-3-phosphocholine; PE, phosphatidylethanolamine; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; NBD, *N*-(4-nitrobenz-2-oxa-1,3-diazole); CF, 5(6)-carboxyfluorescein; DSPE, 1,2-distearoyl-*sn*-glycero-3-phosphoethanolomine; PEG, polyethyleneglycol.

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logical macromolecules, such as albumin, globulin, or chymotrypsin [16].

Among other things, we are systematically developing and optimizing lipid systems for various applications. Here we wish to report our rationale for and our results of the development of the biocompatible – but cholesterol-free – drug-carriers for the systemic applications and sustained drug release in vivo.

Using the basic principles of our rational membrane design we anticipated that by combining relatively compact lipid bilayer interior with a mobile steric surface-barrier one should obtain lipid vesicles which are optimally suited for the long-term circulation in blood and for the sustained drug release under physiological conditions. The rigidity of the carrier membrane should increase the permeability barrier and thus reduce the drug loss. The sterical surface-protection should slow down the adsorption of macromolecules from the blood onto vesicular surface and thus suppress the elimination of the drug-carrying system from blood by the phagocytes.

As an example of suitable lipid composition for the vesicle carriers we have chosen distearoylphosphatidylcholine mixtures with distearoylphosphatidylethanolamine-polyethyleneglycol. In accord with expectation we have discovered that such liposomes stay in blood and avoid liver and spleen for many hours by the virtue of their selected surface characteristics. Our present choice of a neo-phospholipid as a membrane additive simultaneously minimizes the loss of the model drugs from the vesicle interior.

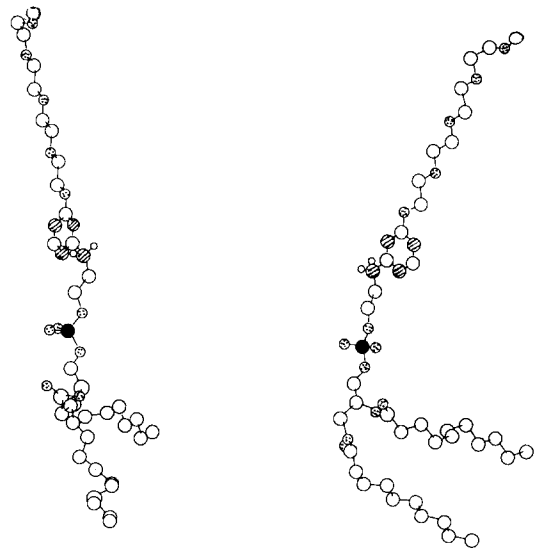

Fig. 1. Molecular structure of the essential parts of neo-phospholipid PE-PEG in orthogonal views. The displayed and modelled part of the polyoxyethylene headgroup section was chosen to be approx. 5% of the actual length (5 segments), so as to represent the average headgroup length assuming an uniform coverage of the liposomal surface by the polymer. To obtain the structural form in space a molecular optimization procedure using MM2 force field has been performed for a reduced-size lipid with two undecanoyl hydrocarbon chains and 20 adjacent water molecules; the latter are omitted in this representation for the sake of clarity. (P, black atoms; O, dotted atoms; N, dashed atoms).

Material and Methods

Chemicals. Distearoylphosphatidylcholine (DSPC) and distearoylphosphatidylethanolamine (DSPE) were obtained from Boehringer, Mannheim (F.R.G.). Their purity was controlled by thin-layer chromatography on silicic acid plates; 1,2-dipalmitoyl-*sn*-glycero-3-[*N*-*methyl*-^{3}H]phosphocholine ([^{3}H]DPPC) was from Amersham Buchler (U.K.), and fluorescently labelled phosphatidylethanolamine with *N*-(4-nitrobenz-2-oxa-1,3-diazole)-headgroup (NBD-PE) from Sigma (U.S.A.). 5(6)-Carboxyfluorescein (CF) was purchased from Eastman Kodak (U.S.A.) and used without any purification. Methoxypolyethyleneglycol 5000 activated with cyanuric chloride was supplied by Sigma (U.S.A.).

Synthesis of DSPE-PEG. Distearoylphosphatidylethanolamine-polyethyleneglycol was prepared by the method of Abuchowski et al. [17] which was originally developed for the chemical modification of bovine serum albumin. This method is based on a single step covalent binding of activated methoxypolyethyleneglycol (PEG) to an amino group, in our case of distearoylphosphatidylethanolamine. In brief, DSPE (0.35 mM) and triethanolamine (1.2 mM) are dissolved in 10 ml chloroform/methanol (5 : 1) and added to a solution of activated PEG (0.4 mM) in 50 ml chloroform/methanol (1 : 5). This mixture is stirred for 5 days at room temperature. Conversion is 100% in terms of DSPE and affords essentially pure product, as concluded from thin-layer chromatography on silic-acid plates with chloroform/methanol/water (50/20/1) mixture as solvent. The corresponding lipid structure (for a reduced-size compound with shorter chains and head) is shown in Fig. 1. It has been optimized by a molucular modelling programme using MM2(77) force field including 20 waters of hydration, which in this picture are not shown.

Stability experiments. A 5 weight-% suspension of DSPC or DSPC/DSPE-PEG mixtures is sonicated by a tip-sonicator in 30 mM carboxyfluorescein solution (pH 7.2) until the average vesicle diameter is approximately 80 nm, as determined by dynamic light scattering on a Zeta-sizer 2 (Malvern, U.K.) equipped with a software for determining continuous vesicle size-distribution profile. To avoid marker leakeage through the membrane imperfections originating from the sonication-defects all vesicle suspensions are aged for at least three days prior to measurements. Un-encapsulated carboxyfluorescein, which at the given concentration is self-quenched, is removed by the gel-filtration through a Sephacryl S400 mini-column. In a typical drug-release assay liposomes (50 μg total lipid) are mixed with 1 ml of prewarmed human plasma containing 0.02 sodium azide and in-

cubated at 37 °C. Release of the model-drug CF increases the dye fluorescence because of the dilution-dependent de-quenching. We monitor this change on a fluorescence spectrometer (LS-5, Perkin-Elmer, U.K.) with excitation at 490 nm and emission wave-length of 520 nm. A 500 nm cut-off filter in the emission path reduces the signal from the light scattered on liposomes. Total amount of the entrapped carboxyfluorescein is calculated after terminal lysis [18] of the liposomes with Triton X100 (1.2 vol% final concentration).

Phagocytosis experiments in vitro. Small unilamellar liposomes containing 2.5 mol% of fluorescently labelled lipid NBD-PE are prepared in 100 mM Hepes buffer (pH 7.2). $1 \cdot 10^6$ cells of a human blood-monocyte line (THP-1, 19) are incubated with 10 μl liposomal suspension (500 μg total lipid). In the first approximation, such incubation at 37 °C in human plasma simulates an intravenous liposome injection. After different periods of time the cells are washed extensively with an isotonic, ice-cold phosphate buffer and the cell-associated liposomal contents are determined by flow-cytometry (FACScan; Becton Dickinson). Specimens kept at 4 °C, where essentially no phagocytosis occurs, are also measured as negative controls.

In vivo experiments. Unilamellar DSPC or DSPC/DSPE-PEG (9/1) liposomes with a diameter of 170 (100) nm and 2.3 mol% of [^{3}H]DPPC (2 μCi/mg total lipid) are prepared in 100 mH Hepes buffer (pH 7.2) as described already for the in vitro experiments. In each individual test, four female, 2-months-old NMRI mice (with a weight of approx. 33 g) are injected with 1.5 mg phospholipid in 0.15 ml suspension through the tail vein (45 mg lipid/kg body weight). At selected times, blood samples (40 μl) are collected by cutting the end of the tail. After 8 or 24 h mice are killed and their liver, spleen, kidney, lung, heart and tigh-muscle tissue are excised. Blood specimens and 100 mg of each collected tissue are decoloured with 0.4 ml H_2O_2 and 0.2 ml $HClO_4$ at 80 °C overnight. Subsequently they are neutralized with 0.2 ml CH_3COOH. After the addition of 10 ml Aquasol-2, total activity of the samples is measured in a beta-scintillation counter (Berthold, Wildbad).

All measured time dependences are numerically fitted to an expression of the form

$$R(t) = k_0 + k_1 \exp(-t/\tau_1) + k_2 \exp(-t/\tau_2)$$

In most cases, however, it turnes out that the temporal signal decay or increase is monoexponential. The appropriate optimal parameter values are given in the text.

Results

Liposome characterization

Immediately after sonication, all liposomes investigated in this study had a diameter of approx. 80 nm, as concluded from dynamic light scattering, by the choice of our specific experimental conditions. From electron micrographs we conclude that they were predominantly unilamellar. Three days later, the size of the vesicles made of DSPC was greater by 215 or 175% and the radius of DSPC/DSPE-PEG liposomes greater by 20 and 5% in the buffered saline and 30 mM CF solution, respectively (see Table I). We believe this difference to be due to the fact that carboxyfluorescein renders bilayers slightly electronegative, more so for pure phosphatidylcholine than for PEG-coated phosphatidylcholine vesicles. Zeta-potential measurements show this directly (Table I). From similar data for DSPE-PEG-containing vesicles we infer that this lipid contributes a minute positive charge to the total bilayer potential. Despite all this, lipid vesicles used in this study were fairly electroneutral. The magnitude of their zeta-potentials, as measured by doppler laser anemometry, were not higher than 10 mV (Table I).

Supplementing phosphatidylcholine bilayers with a small amount of DSPE-PEG caused the liposomal

TABLE I

Initial diameter, size-increase, zeta-potential and the efficiency of drug encapsulation into distearoylphosphatidylcholine and various distearoylphosphatidylcholine/distearoylphosphatidylethanolamine-polyethyleneglycol mixed vesicles

Composition (molar ratio)	Diameter (nm)	Size-increase (relative)	Zeta-potential (mV)	Encapsulation (%)
Phosphate-buffered saline (PBS):				
DSPC	78 ±1 [a]	2.14±0.24	−3.5±0.5	
DSPC/DSPE-PEG (9.0:1.0)	86.5±9	1.20±0.16	−1 ±0.1	
30 mM carboxylfluorescein in PBS:				
DSPC	86.5±7.5	1.76±0.21	−6 ±0.2	3.8±0.3
DSPC/DSPE-PEG (9.75:0.25)	70.5±1	1.01±0.09		9.4±0.5
DSPC/DSPE-PEG (9.5:0.5)	83 ±3.5	0.98±0.08		9.7±0.3
DSPC/DSPE-PEG (9.0:1.0)	84 ±5.5	1.06±0.1	−1 ±0.1	5.6±0.3

[a] Every result is a mean of at least five independent measurements.

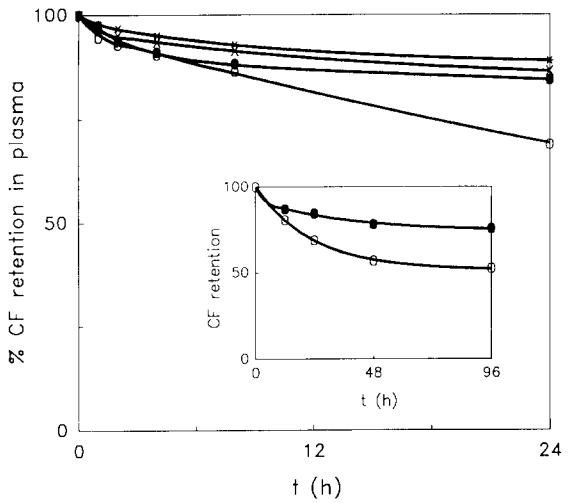

Fig. 2. Time-course of the plasma-mediated release of a model drug carboxyfluorescein from various lipid vesicles at 37 °C. Inset shows similar results measured over four days. Liposomes containing neophospholipids with PEG-heads in increasing molar ratios (pure DSPC (○); DSPC/DSPE-PEG (9.75 : 0.25, *); DSPC/DSPE-PEG (9.5 : 0.5, ×) and DSPC/DSPE-PEG (9 : 1, ●)) were incubated in human plasma at 37 °C. The release of carboxyfluorescein from such unilamellar or oligolamellar vesicles was determined fluorimetrically as described in the text. Fitting parameters pertaining to the single-exponent curves are given in the main text body.

surface to become (partly) covered by a layer of polyethyleneglycol headgroups. This hampered the interliposomal fusion and prevented quite efficiently an increase of the vesicle radius for PEG-coated carriers. We found that this effect was independent of DSPE-PEG concentration in the lipid bilayers in the range between 2.5 and 10 mol%.

The encapsulation rate of DSPC/DSPE-PEG mixed vesicles exhibited an optimum when the concentration of the minor component was close to 5 mol%. With 10% carboxyfluorescein inside vesicles the encapsulation efficiency in the latter case was by approx. a factor of three higher than for pure distearoylphosphatidylcholine (cf. Table I).

Drug release in plasma

In order to optimize the liposome-mediated drug release and to support drug-retention in carrier vesicles we had used liposomes with bilayers which under physiological conditions are in the ordered, gel-phase. As desired and expected, this ensured the permeation-mediated loss into plasma of the carboxyfluorescein entrapped into distearoylphosphatidylcholine vesicles to be less than 25% over a period of 24 h. Addition of DSPE-PEG to distearoylphosphatidylcholine brought further amelioration and reduced this loss to a value below 15% per day. Data presented in the insert to Fig. 2 indicate that such loss reduction persists for at least 4 days.

Uptake of liposomes by phagocytes

For a general drug delivery it is desirable that liposomes should stay in blood for long time. Normally, however, all liposomes are rapidly recognized as 'foreign', mainly by the cells of the mononuclear-phagocyte system. They are, consequently, taken-up by these cells and digested or stored in the lysosomes, depending on the total lipid concentration.

The primarily reason for such liposome recognition and elimination is adsorption and subsequent (partial) denaturation of certain macromolecules (such as fibronectin, albumin, C-reactive protein, etc.) on and in the lipid bilayers. But peculiarities of the liposome composition also are relevant. Most of the standard systemically applied lipid vesicles, therefore, are cleared from blood by the endocytically active cells and find their end in the phagocyte-reach organs.

To investigate this process in vitro we have been monitoring the liposome uptake into the phagocytically active cells in a cell culture. Our assay is based on an incubation of fluorescently labelled liposomes with a fixed number of cells of the blood monocyte line THP-1 (19) in human plasma, followed by a determination of the intracellular fluorescence in a flow-cytometer as a function of time. Cell-associated fluorescence corrected for the nonspecific adsorption is a direct measure of the phagocytosis rate.

In our present experiments the vesicle uptake was typically followed over a period of six hours (Fig. 3). After coating the liposomal surface by a layer of polyethyleneglycol coupled to DSPE the vesicle uptake by monocytes was suppressed ($\tau = 33$ h) relatively to the corresponding uptake of standard distearoylphosphati-

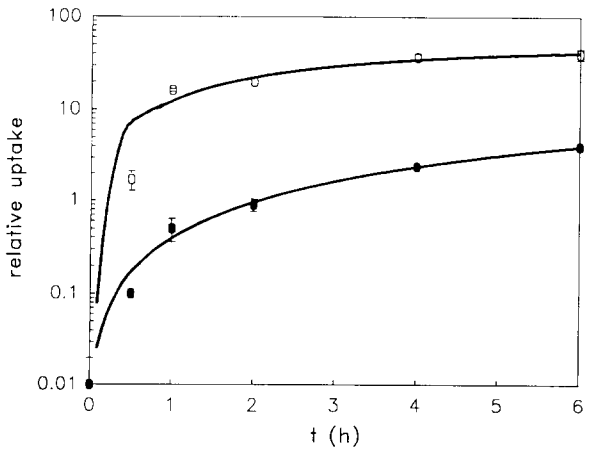

Fig. 3. Liposome uptake by THP-1 (human monocyte) cells in human plasma at 37 °C as a function of time. The rate of the phagocytotic uptake of DSPC (○) and DSPC/DSPE-PEG (9 : 1) (●) middlesized liposomes (1 mg phospholipid/ml human plasma) by 1 million of THP-1 cells was determined as described in methodological section. Curves were calculated by assuming mono-exponential uptake rates $\tau_1 = 3$ and $\tau_1 = 33$ h, respectively. Where no error-bars are seen standard deviation is smaller than the data points.

dylcholine vesicles. At 37°C, in a rule, only the latter type of vesicles was phagocytosized at a notable rate ($\tau = 3$ h). By comparing the data pertaining to the physiological 37°C and the data collected at 4°C, where phagocytosis was nearly completely prevented, we conclude that the relative proportion of the vesicle adsorption vs. vesicle phagocytosis was 0.6 for DSPC and 0.93 for DSPC/DSPE-PEG liposomes (not shown).

In vivo results

Results of animal experiments confirm the significance of our in vitro data. Vesicles coated with polyethyleneglycol-5000 were removed from blood at only 15% of the pace characteristic for distearoylphosphatidylcholine liposomes. The clearence rate of the former vesicles within the first 8 h after i.v. application was $\tau = 8.4$ h and first-order with an asymptotic value of 25%. Consequently, even after a period of 24 h, approx. 1/4 of the applied dose was still in circulation. It is noteworthy that by including the 'constant' (this is, the very long-lived) component into a less meticulous data analysis the clearance rate would far exceed 20 h, the latter value characterising the optimal range achieved by other authors to date. Our liposomes are thus, indeed, unprecedentedly well suited for the sustained drug delivery.

The elimination of pure phosphatidylcholine liposomes was much faster and occurred on a characteristic time scale of $\tau = 0.47$ h h (Fig. 4). The long-term dose in blood this time was only 9%.

The distribution pattern of standard and sterically stabilized lipid vesicles in various tissues 8 hours after

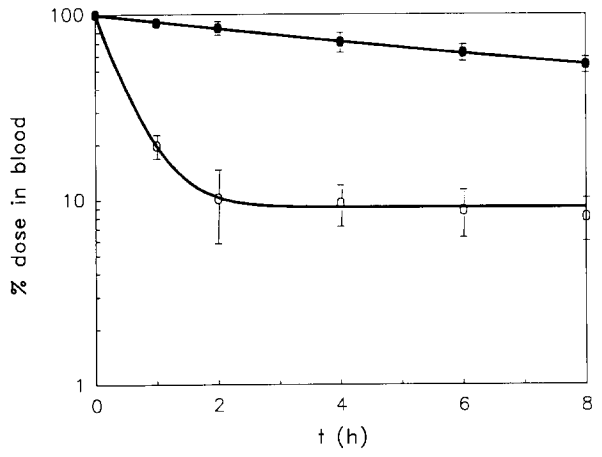

Fig. 4. Liposome clearence from blood as a function of the time after an intravenous administration in mice. Data points give the relative amount of the liposome-associated dose in the murine bloodstream for DSPC (○) and DSPC/DSPE-PEG (9:1) (●) tritium-labelled liposomes (45 μg phospholipid/g body weight). Each value is an average of four independent experiments. Liposome elimination is exponential on a time scale of $\tau_1 = 0.47$ h and 8.4 h for the former and latter liposome types, respectively, with $\tau_2 = 0$ in both cases; the corresponding 'steady-state', limiting vesicle concentrations (k_0) are 9% and 25%, respectively.

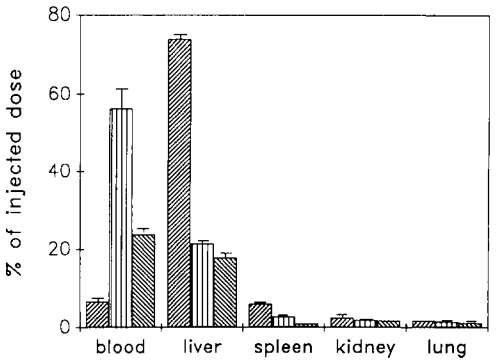

Fig. 5. Biodistribution of the standard (DSPC, right dashed) and long-lived (DSPC/DSPE-PEG, vertically dashed) liposomes after an intravenous administration in mice. Tissue distribution of the radioactivity from tritium labelled DSPC and DSPC/DSPE-PEG (9:1) liposomes 8 h after an application of 1.5 mg phospholipid per mouse is shown as right dashed and vertically-dashed boxes, respectively. The last (left dashed) set of boxes illustrates the distribution of the radioacativity from DSPC/DSPE-PEG (9:1) vesicles 24 hours post injectionem.

an intravenous application is displayed in Fig. 5. In contrast to pure DSPC liposomes, the DSPE-PEG-containing liposomes are seen to be taken-up by the phagocyte-reach organs less avidly, the relative concentrations of the labelled lipids in liver and spleen for the two liposome classes being 80 and 24.5%, respectively. Also the blood-to-liver ratio reflects this trend. The value of this ratio for phosphatidylcholine liposomes is 0.08 after 1/3 of a day. Conversely, 8 h post injectionem we regularly find that 2.3-times more DSPC/DSPE-PEG vesicles was in blood than in the liver.

Independent of the vesicle composition less than 5% of the total dose are recovered in the lung. This shows that our liposomes are hardly filtered and eliminated by this organ. Total amount of the liposome-associated radioactivity detected in heart and other muscle tissues is even lower, less than 0.8%. The concentration of labelled lipids in the brain is, as one would also expect, quite low, less than 1.5% of the total dose.

All investigated organs and tissues are morphologically unaffected by the liposome application.

Discussion

The clearance rate and the tissue distribution of intravenously injected liposomes, on the one hand, depend on the vesicle size distribution [19]. On the other hand, the vesicle charge is believed to play a role. Positively charged or neutral [19–21] small liposomes [22–24] have been reported to stay in blood longer than large and/or anionic vesicles; if negatively charged lipids must be used, shielding of the negative charge should be of help [12–15]. Our results show that the situation is more complex and that other aspects must

also be considered; lipid charge, by no means is the main determinant of the vesicle uptake (unpublished data).

By mixing distearoylphosphatidylcholine and cholesterol in a 1 : 1 molar ratio Judith Senior and G. Gregoriadis have prepared liposomes which are nearly impermeable for the water soluble substances, such as carboxyfluorescein, and may circulate in blood for quite long times after a systemic application. Obviously, such vesicles are better suited as carriers than most of the standard liposomes. But they also have a disadvantage in that they contain a very – even excessively – high amount of cholesterol. This makes them unsuitable for certain applications, for example in patients with a high risk of hypercholesterimia. Moreover, such cholesterol-rich vesicles are also extremely difficult to make in large quantities.

Based on our data, we conclude that lipid vesicles in the gel phase (far enough from the chain-melting phase transition) can, but need not, be stabilized additionally against the loss of encapsulated water-soluble drugs.

The situation is different when both drug retention and carrier concentration (liposome circulation time) in blood are considered. Most lipid vesicles fail to stay in blood for more than a few hours, owing to the opsonic activity of the plasma components and to the liposome removal by the cells of the mononuclear-phagocytic system. If lipidic carriers are to be used for the sustained drug release or long term action precautions must, therefore, be met to suppress such lipid vesicle elimination by the phagocytes.

The first promising results in this direction were achieved with sterically stabilized microspheres [10]. The first attempts to construct sterically stabilized, long-lived liposomes for the use as drug-carriers in the sustained drug release were, therefore, based on similar considerations. They led to encouraging results. But their basic components were phosphatidylcholine and different sugar-containing compounds [12–15]. The latter gave rise to a steric surface barrier but could, in principle, be immunologically dangerous *. It is consequently unclear whether or not such liposomes will find their way into applications as common drug carriers owing to the potential immunological side effects, such as anti-carbohydrate antibody formation, but also because of the high manufacturing costs.

To avoid such difficulties and hazards we had deliberately chosen a non-glycosidic lipid as a stabilizer. We have used a mixture of phosphatidylcholine with a neo-phospholipid as our first example of carbohydrate- and cholesterol-free long-lived liposomes.

On the one hand, this ensured the vesicles to stay in blood longer than any other lipidic carriers explored so far. On the other hand, our choice had to do with the potential use of liposomes in medicine and biology. We want our liposomes to have as few adversary side-effects as possible and believe that neolipids containing polymeric head-groups are very interesting in this respect. Firstly, many polymers are non-immunogenic. Secondly, numerous polymeric substances are also little toxic or non-toxic. Indeed, concluding from the available toxicological data on PEG-containing detergents [25] we infer that even our present liposomes – or their variants – should be usefull for the therapeutic use in vivo.

The main advantage of DSPC/DSPE-PEG vesicles and related liposomes is their long circulation-time in blood. This results, in part, from the limited accessibility of the hydrophobic binding sites in the highly compact lipid bilayers. Binding sites for the blood macromolecules are not accessible on the vesicle surface and thus are cryptic. The induction of phagocytosis is thus delayed and the vesicle circulation prolonged. Moreover, all lipid components of our liposomes have homologous, long chains which are in the gel phase under the physiological conditions. The danger of lateral phase separation and the formation of protein binding-sites at the phase or domain boundaries is thus minimized. The other reason for the resistance of our liposomes to phagocytosis is the presence of the sterical surface-barriers on the vesicles which are created by the PEG residues attached to phosphatidylethanolamine. Let us estimate the lower limit for the thickness of this barrier. To simplify the calculation we assume that the PEG-headgroups are uniformly distributed over both bilayer surfaces and fill-out uniformly the innermost part of the interfacial region. We know that PEG segments are disordered under our experimental conditions and that the molecular mass of each headgroup is approx. 5000 Da. From all this, one can conclude that the surface region inaccessible to the water soluble proteins, on the average, is approx. 2 nm thick. This value pertains to DSPC liposomes with 10% DSPE-PEG.

The mechanism used in this work to prolong the liposome circulation-time in blood is rather unspecific. Its validity, therefore, should not be limited solely to the present chemical form of the stabilizing neo-lipid or to the PEG-containing bilayers alone. In principle, any polymer with similar physico-chemical properties and low biological reactivity could be used for the same purpose with comparably good chances for attaining useful liposomes. It is even likely that by using different headgroup lengths, diverse headgroup types, or mixtures of several sterically active components we will

* Despite the fact that natural glycolipids are normally non-immunogenic, it is possible that upon inclusion of such components into artificial membranes at concentrations higher than biological and in combinations not encountered in the living systems sugar residues might become immunogenic. Results obtained under pathological conditions suggest this.

prepare lipid vesicles which, from one point of view or another, will be even better than the ones introduced in this article. Obviously, for any new liposomal composition or formulation additional (re)optimization will be required. The time demand for such work can be kept at minimum, however, if certain basic rules of the rational membrane design are obeyed, some of which had been discussed in this work. Then – and only then – new liposome types for the sustained drug release or for the site-specific drug delivery will be soon within reach. Sterically stabilized immunoliposomes will, hopefully, follow.

The concepts described in this work should also prove valuable for the design of improved formulations for the parenteral nutrition and other systemic applications.

In summary, we have used in vitro and in vivo test-systems to study the uptake of lipidic drug-carriers by the phagocytic cells. Our cell-experiment data are in good agreement with the results of in vivo experiments and show that liposomes with compact bilayers can ensure high retention of the water soluble substances in plasma. This justifies future tests with such drug-carriers both in vitro and in vivo.

Additional creation of the steric barriers on the carrier-vesicle surface was shown to prolong dramatically the vesicle circulation times and guarantee a relatively high blood concentration of the drug-carrying liposomes over a period of at least 24 h. New perspectives for the use of liposomes in medical applications, most notably for the sustained drug release in blood and for the parenteral applications, emerge from this.

Acknowledgements

We are thankful to Mrs. S. Siebels for her friendly and committed technical assistance. This study had been supported in part by the Deutsche Forschungsgemeinschaft through grants Ce 19/1-1 and SFB 266/C9. The use of the Central Animal Facilities of Gesellschaft für Strahlenforschung, Neu-Herberg b. M., is gratefully appreciated.

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