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Short communication

Influence of lipopolymer concentration on liposome degradation and blood clearance

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Abstract

It is well known, that a prolonged liposome circulation time can be achieved by incorporation of lipopolymers into the lipid membrane thereby reducing interactions with destabilizing factors in the blood stream, e.g. phagocytic cells and lipoproteins. However, very little is known about the enzymatic degradation of steric hindered liposomes introduced into body fluids. In this study, the blood clearance and the PLA_2 catalyzed degradation of unilamellar dipalmitoylphosphatidylcholine (DPPC) liposomes incorporated with increasing amounts of dipalmitoylphosphatidylethanolamine–polyethyleneglycol (DPPE-PEG), was investigated. The results demonstrated an increase in PLA_2 activity for increasing amounts of lipopolymer in the lipid membrane, while the liposome blood clearance was prolonged by incorporation of DPPE-PEG into the liposomes. Hence, these results suggest that it may be possible for long circulating liposomes to obtain a site specific liposome degradation and release of drug substance in tissue with high levels of PLA_2. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Lipopolymer concentration; Liposome degradation; Blood clearance

1. Introduction

Phospholipid liposomes introduced into the bloodstream are usually exposed to a rapid degradation and/or removal from the blood stream. Different liposome degradation mechanisms exist in the body. The main reason for removal of conventional liposomes from the blood stream is the rapid uptake by the macrophages of the reticuloendothelial system (RES) (Woodle and Lasic, 1992). However, this problem can be overcome by incorporation of the lipopolymer, phosphatidylethanolamine–polyethyleneglycol (PE-PEG) into

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the liposomes (Senior, 1987; Blume and Cevc, 1993). It is believed that the steric barrier prevents recognition and uptake by the macrophages (Blume and Cevc, 1993; Lasic and Martin, 1995).

Another pronounced degradation mechanism is the release of phospholipid components of the liposome caused by interactions with lipoproteins, red blood cells and endothelial cell membranes (Senior, 1987; Bach et al., 1996). Interaction between liposomes and lipoproteins and/or cells may promote phospholipid transfer to—and exchange between—these blood components. It has been shown that steric hindrance of the PEG-liposomes caused a reduced adsorption of plasmaproteins to the liposomes and an increase in the circulation time (Blume and Cevc, 1990; Chonn and Cullis, 1995).

The enzymatic degradation of liposomes in the circulation is probably only of minor importance, however, the liposomes are potential substrates for several of the blood enzymes, e.g. lechithin–cholesterol–acyltransferase (LCAT), lipoprotein lipase, hepatic lipase and the specific phospholipases, e.g. PLA_1, PLA_2, PLC, PLD (Senior, 1987; Bach et al., 1996). Therefore, the presence of high levels of these enzymes may be important in liposomal degradation. PLA_2 belongs to a group of membrane active enzymes which are present in extra- and intracellular compartments of human tissue (Vance and Vance, 1991; Hansen et al., 1995). In particular, high concentrations of PLA_2 are found in extravascular pathological tissues (Jain and Berg, 1989; Gelb et al., 1994).

In order to gain further insight into the different liposome destabilization mechanisms due to tissue specific differences in enzymatic activities, the blood circulation time and the PLA_2-catalyzed hydrolysis of dipalmitoylphosphatidylcholine (DPPC) liposomes incorporated with different concentrations of dipalmitoylphosphatidylethanolamine–polyethyleneglycol (DPPE-PEG_{2000}) has been studied.

2. Method

Multilamellar radioactive liposomes composed of DPPC incorporated with submicellar DPPE-PEG_{2000} concentrations were prepared by dissolving and mixing the phospholipid and lipopolymer in chloroform. [^{14}C]DPPC was used as a radioactive marker. Chloroform was removed by an air stream and dried overnight at low pressure. The multilamellar liposomes were hydrated in buffer for at least 1 h at 51°C. Unilamellar liposomes with a size of 100 nm were made from the multilamellar liposomes by extrusion through 100 nm polycarbonate filters. Size measurements were performed by dynamic light scattering using a Zetasizer 4 (Malvern, UK). The unilamellar liposomes were used for the PLA_2 catalysis. Assay conditions for the PLA_2 catalyzed phospholipid hydrolysis included 2 mM unilamellar liposomes, 0.49 μM PLA_2, 10 mM $CaCl_2$, 135 mM KCL, 9 mM Hepes (pH 7.5) and 0.9 μM EDTA. The liposomes were incubated in buffer with phospholipase A_2 for 20 min at 41°C. The reaction was stopped by 0.5 ml chloroform methanol acetic acid (2:4:1 v/v/v). Hereafter, the lipids were extracted by chloroform, separated by TLC and the radioactivity of the individual lipid spots was determined by liquid scintillation counting.

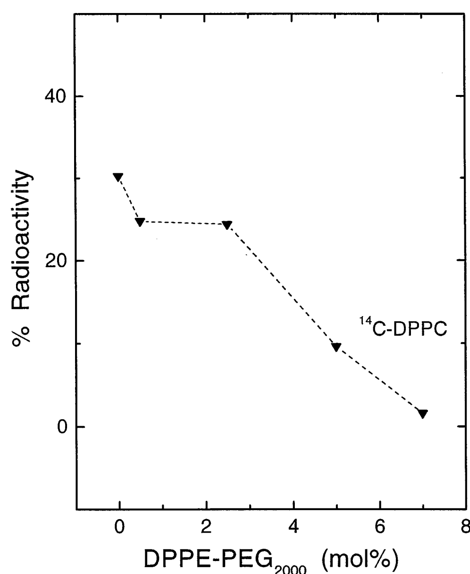

Fig. 1. PLA_2-hydrolysis of DPPE-PEG_{2000} containing DPPC-liposomes at 41°C as determined by percent of radioactive phospholipids ([^{14}C]DPPC) 20 min after adding the enzyme to the liposome suspension ($n = 6$).

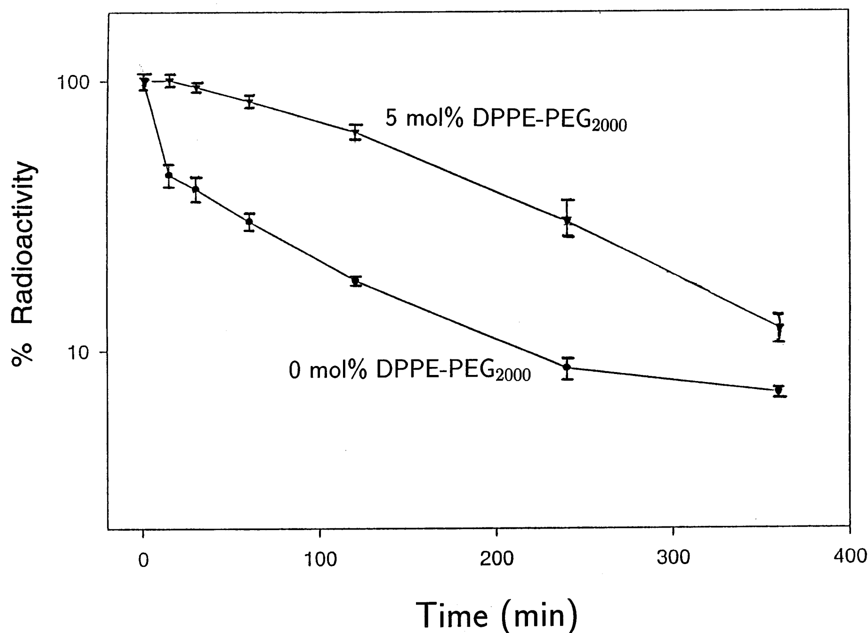

Fig. 2. Clearance of DPPE-PEG$_{2000}$ containing DPPC-liposomes from the blood-circulation in mice compared to clearance of pure DPPC-liposomes ($n = 5–6$). Data points give the relative amount of the i.v. administered lipid-associated radioactivity in the blood as a function of the time. Upper curve: DPPE-PEG$_{2000}$ containing DPPC liposomes; lower curve: pure DPPC liposome.

For the blood clearance study, radioactive DPPC liposomes containing 0–7 mol% DPPE-PEG$_{2000}$ were i.v. injected into the tail vein of mice. The injected dose corresponded to 1.5 mg DPPC, 2.5 μCi [^{3}H]DPPC per 30 g body weight. Blood samples were taken at different time points: 15, 30, 60, 120, 240, and 360 (min) and they were bleached before liquid scintillation counting.

3. Results and discussion

The size measurements showed, that during 30 days of storage the PEG-liposomes retained their originally size due to steric hindrance of the liposome membrane by the incorporated lipopolymers. In contrast, the pure DPPC liposomes showed a minor increase in size from 100 nm to approximately 125 nm during the first week of storage, probably due to fusion and/or aggregation of the particles.

The PLA$_2$-induced phospholipid hydrolysis showed a concentration dependent increase in PLA$_2$ activity for increasing amounts of lipopolymer in the liposome (Fig. 1). Incorporation of 7 mol% DPPE-PEG$_{2000}$ into the liposome bilayer caused a complete PLA$_2$-catalyzed hydrolysis of the lipids after 20 min (Fig. 1). The blood clearance study showed a significant prolongation of the circulation time of PEG-liposomes compared to pure DPPC liposomes as shown in Fig. 2. However, no significant effect of DPPE-PEG$_{2000}$ concentrations in the range of 2.5–7 mol% on the blood clearance was seen.

These results suggest, that liposomes containing DPPE-PEG in submicellar concentrations promote PLA$_2$-induced degradation of the phospholipid membrane. Incorporation of PEG lipids into lipid membranes may cause a heterogeneous bilayer structure with lipid packing defects, lipid domains and phospholipid interfacial regions, which may promote the activity of the interfacial active PLA$_2$ (Op den Kamp et al., 1974; Hønger et al., 1997). In addition, the hydrophilicity of the lipopolymers may increase the binding and the contact time of the small water soluble enzyme to the liposomes.

It is well known, that a prolonged liposome circulation time can be achieved by incorporation of lipopolymers into the bilayer (Woodle and Lasic, 1992; Sharma and Sharma, 1997) probably due to reduced interaction with phagocytic cells and plasma proteins (Sharma and Sharma, 1997). The hydrophilic polymers form a steric barrier, which repel opsonin penetration and adsorption, and decrease the uptake by macrophages of the reticuloendothelial system. As generally observed, the PEG-liposomes in this study showed a prolonged circulation time compared to the conventional DPPC liposomes.

Hence, it may be possible using long circulating liposomes to obtain a site specific release of liposomal encapsulated drug substance in tissues with a high PLA_2 level, e.g. in inflammatory tissue, by adjusting the PEG content (Vermehren et al., 1998). In this study, an increase in DPPE-PEG content probably caused an increase in liposome-PLA_2 interaction. This is in contrast to the generally observed reduction in plasmaprotein adsorption to PEG-liposomes.

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