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Rapid Report

Amphiphilic vinyl polymers effectively prolong liposome circulation time in vivo

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Abstract

Newly synthesized amphiphilic polyacrylamide and poly(vinyl pyrrolidone), single terminus-modified with long-chain fatty acyl groups, are able to incorporate into the liposomal membrane, and similar to poly(ethylene glycol) prolong liposome circulation in vivo and decrease liposome accumulation in the liver. Protective efficacy of modified polymers increases with the increase in the length of acyl moiety and decreases for higher molecular weight polymers. The data on amphiphilic polymer-modified liposome biodistribution are presented.

Keywords: Drug carrier; Long-circulating liposome; Amphiphilic polymer; Polyacrylamide; Poly(vinyl pyrrolidone); Biodistribution

Poly(ethylene glycol) (PEG)-protected liposomes are now very popular objects within the liposome research area [1–3]. The ability of PEG-liposomes to circulate long in vivo has made them promising drug carriers. Starting from the first description of such liposomes [4], the mechanism of PEG protective action is under continuos investigation [5–7]. With the understanding of this mechanism some other polymers might probably be suggested for liposome protection in vivo, widening thus possible areas of biomedical application of liposomes.

Recently, we have suggested a hypothetical model of PEG behavior on the liposome surface [8,9], based on the statistical properties of polymer molecule in solution. According to this model, the molecular mechanism of PEG protective action is determined by the properties of a flexible polymer molecule (free rotation of individual units around inter-unit linkages) in solution, and includes the formation of dense 'statistical cloud' of possible polymer conformations over the liposome surface even at relatively low polymer concentrations. This 'cloud' prevents liposome surface from the interaction with plasma proteins and opsonization which is believed to be the main reason of liposome capture by the liver [10]. A more rigid polymer fails to form this dense protective cloud, even when hydrophilic. The model formulates also some general requirements towards polymers which can be used for liposome steric protection: (a) polymers should be soluble and hydrophilic, and (b) have highly flexible main chain. Polymer biocompatibility has to be added to the list, if polymer-liposomes are intended for medical use. Polyacrylamide (PAA), poly(vinyl pyrrolidone) (PVP), and poly(vinyl alcohol) were named as the most appropriate candidates among alternative liposome protectors.

Here we report the first experimental data on possible use of synthetic polymer other than PEG for steric protection of liposomes and preparation of long-circulating liposomes. Amphiphilic PAA and PVP have been used in our studies.

Both PAA and PVP were prepared by radical polymerization of monomers in dioxane (monomer concentration was 10% wt. for acrylamide and 50% wt. for

Abbreviations: PEG, poly(ethylene glycol); PAA, polyacrylamide; PVP, poly(vinyl pyrrolidone); HBS, Hepes-buffered saline; M_r, molecular weight.

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vinylpyrrolidone) in the presence of 1% wt. of 2,2'-azobisisobutyronitrile for 6 h at 70°C (all reagents from Sigma, USA). Using different quantities of growing chain terminator, both polymers were prepared with M_r values 6000–8000 (low-molecular-weight polymers, PAA-L and PVP-L) and 12000–15000 (high-molecular-weight polymers, PAA-H and PVP-H), as determined by viscosimetry and gel-chromatography on Sephadex G-25 with standards. Polymers prepared have been made amphiphilic and membranotropic by chemical attachment of hydrophobic acyl groups of different length to a single terminus of a polymer molecule. The following products have been prepared: PAA-L with terminal dodecyl group (PAA-L-D); PAA-L with terminal palmityl group (PAA-L-P); PAA-H with terminal palmityl group (PAA-H-P); PVP-L and PVP-H with terminal palmityl group (PVP-L-P and PVP-H-P, respectively). Control sample of PEG(6000)-phosphatidylethanolamine (PEG-PE) was obtained as in [4].

Liposomes have been prepared by the detergent (octyl glycoside) dialysis method from the mixture of egg phosphatidylcholine and cholesterol (7:3, molar ratio) with the addition, when necessary, of 2.5 or 6.5% mol of the corresponding amphiphilic polymer. Besides, 1% mol of diethylene triamine pentaacetic acid-stearylamine prepared as in [11] has been added to all lipid compositions for the subsequent liposome labeling with ^{111}In. Lipid mixture was argon-dried, vacuumed, solubilized with octyl glycoside in HBS, pH 7.4 (final total lipid concentration ca. 5 mg/ml), and dialyzed overnight against HBS at 4°C. Liposomes obtained were sized by passing through the polycarbonate filters 0.6, 0.4 and 0.2 μm (Nuclepore). The final liposome size in all preparations was between 165 and 190 nm with narrow size distribution (95% of liposomes within $\pm$15 nm interval) as determined with Coulter N4 MD Submicron Particle Size Analyzer (Coulter Electronics). For the labeling with ^{111}In (NEN) via transchelation mechanism, liposomes were incubated for 1 h with citrate complex of ^{111}In at room temperature and than dialyzed overnight against HBS at 4°C to remove free label.

For biodistribution experiments, BALB/C mice were injected via the tail vein with 150 μl of liposome suspension in HBS (ca. 700 μg of total lipid and 1 to 2 μCi of ^{111}In radioactivity). Upon certain time intervals mice have been sacrificed by cervical dislocation, blood and organs of interest were collected, and their radioactivity was meausred in LKB gamma-counter. Four or five animals per each time point were used.

The data presented in Figs. 1 and 2 clearly demonstrate that amphiphilic derivatives of PAA and PVP can provide effective protection to liposomes in vivo similar to PEG, which agrees well with our theoretical considerations [8,9]. The extent of protective activity for different polymers depends on several factors, such as the length of hydrophobic anchor, polymer molecular weight (i.e., chain length and the energy of molecular motion), and the quantity of protecting polymer on the liposome surface. Fig. 1A shows that when modified with the same palmityl residue, PAA, PVP, and PEG of similar molecular weight (ca. 6000–8000) being used in similar concentration, all provide efficient steric protection for liposomes and noticeably increase the residence time of liposomes in the circulation. Half-clearance times for PVP-L-P-, PAA-L-P-, and PEG-liposomes with 2.5% mol content of protective polymer are ca. 45, 80, and 80 min, and for PVP-L-P-, PAA-L-P-, and PEG-liposomes with 6.5% mol content of protective polymer – ca. 120, 140 and 170 min, respectively,

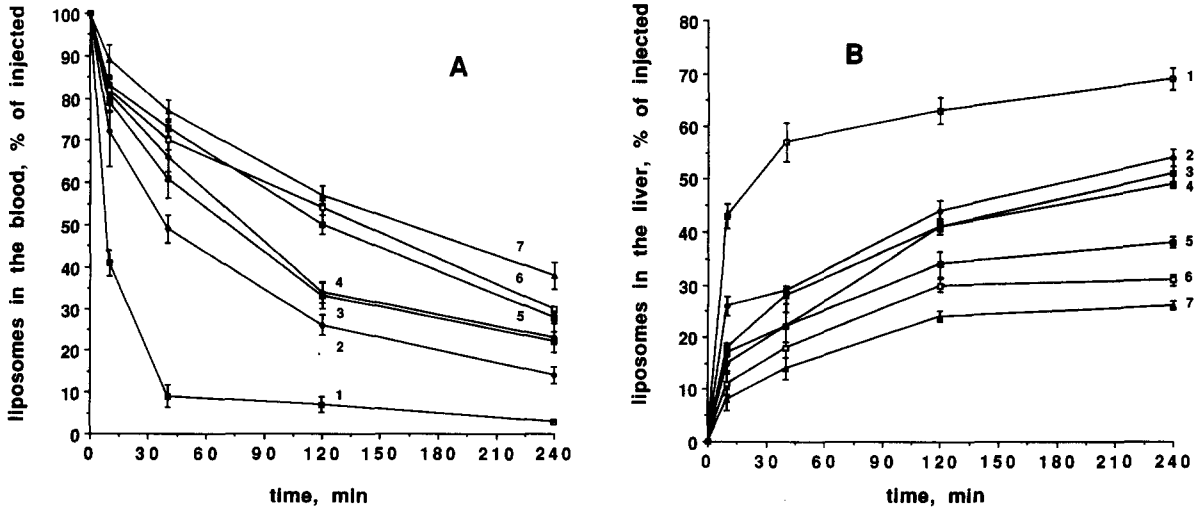

Fig. 1. (A) Liposome clearance from the blood of experimental mice; (B) liposome accumulation in the liver. 1, 'plain' liposomes; 2, PVP-L-P-liposomes (2.5% mol PVP); 3, PAA-L-P-liposomes (2.5% mol PAA); 4, PEG-liposomes (2.5% mol PEG); 5, PVP-L-P-liposomes (6.5% mol PVP); 6, PAA-L-P-liposomes (6.5% mol PAA); 7, PEG-liposomes (6.5% mol PEG).

whereas half-clearance time for 'plain' liposomes of the same size is only about 10 min.

The protective activity of PAA-L-D, and both PAA-H-P and PVP-H-P is, however, much lower (see Fig. 2). Despite of definite increase in the circulation time and some decrease in the liver capture of liposomes (data not shown), these polymers are still much less effective steric protectors than polymers of similar molecular weight, but with longer acyl anchor (compare PAA-L-D- and PAA-L-P-liposomes), or polymers with the same long acyl anchor, but with smaller molecular weight of hydrophilic moiety (compare PAA-H-P-and PVP-H-P-liposomes with PAA-L-P, PVP-L-P, and PEG-liposomes). This can be easily understood taking into account the supposed energy of interaction between the fatty acyl anchor which keeps polymer on the liposome surface and hydrophobic part of the liposomal membrane. From the thermodynamic point of view, relatively short dodecyl group is unable to keep 6–8 kDa polymer molecule on the liposome surface: the energy of the polymeric chain motion is, probably, comparable (or even higher) with the energy of dodecyl group interaction with phospholipid surroundings within the liposomal membrane. As a result, PAA-L-D might be relatively easily removed from the liposomal membrane, and demonstrates only slight and transient protective effect. The longer palmityl anchor provides much more firm polymer binding with liposome (higher energy of interaction with hydrophobic membrane core due to the larger number of membrane-embedded CH_2-groups), and thus much better liposome steric protection (Fig. 1A). On the other hand, even the length of palmityl anchor might be insufficient to provide firm fixation of 12–15 kDa polymer on the liposome surface because of much higher energy of polymer chain motion in solution compared with that for the shorter polymers [12]. So, the liposome surface might gradually loose protective polymer coat; as a result the liposome might be opsonized and captured by the liver and spleen.

Similar regularities have been found following liposome accumulation in the liver (Fig. 1B). Plain liposomes are captured by the liver very fast (more than 50% in 45 min and ca. 70% in 240 min). The longest circulating liposomes containing 6.5% mol of PAA-L-P, PVP-L-P, or PEG demonstrate much slower liver uptake: less than 20% of these liposomes are captured in 45 min, and less than 40% – in 240 min. Liposomes with 2.5% mol of protective polymer demonstrate intermediate liver uptake.

Thus, the decrease in protecting polymer content, the increase in the molecular size of protective moiety, and shortening of the hydrophobic anchor facilitate liposome clearance, which can be easily understood in terms of the density of protective polymer grafting on the liposome surface, and interaction energy between

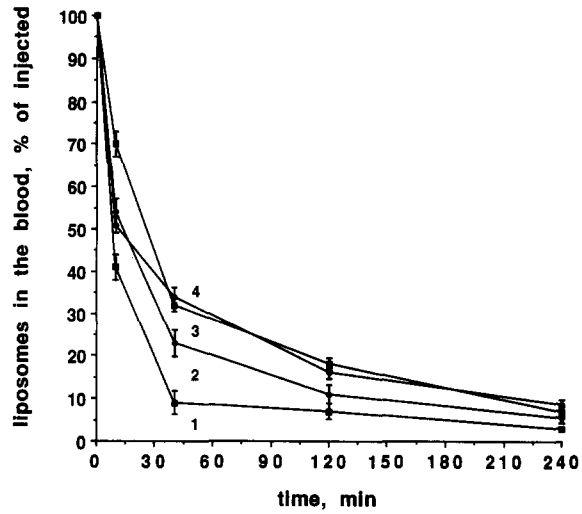

Fig. 2. Liposome clearance from the blood of experimental mice. 1, 'plain' liposomes (reference curve); 2, PAA-L-D-liposomes (2.5% mol PAA); 3, PVP-H-P-liposomes (2.5% mol PVP); 4, PAA-H-P-liposomes (2.5% mol PAA).

grafted polymer and liposomal membrane. Still, the blood clearance and liver accumulation of all polymer-modified liposomes in the liver proceeds slower than for 'plain' liposomes.

The general biodistribution pattern for PAA- and PVP-liposomes does not reveal any specific peculiarities compared with PEG presence on the liposome surface. Spleen accumulation for all coated and non-coated liposomes depends slightly on the nature of protecting polymer but after 4 h always stays within 3 to 10% of injected dose limit. It is interesting to note that being expressed as % injected dose per g of tissue, spleen demonstrates the maximal liposome capture for all types of liposomes and in 4 h upon injection may vary from 50 to 150% dose/g tissue. Other investigated organs – kidneys, lungs, bone marrow, and brain – demonstrated minor liposome accumulation; and variations in biodistribution of different liposomes do not give sufficient data for discussion (data not shown).

In conclusion, we succeded to demonstrate that, in agreement with our previous assumption [8,9], hydrophilic and flexible synthetic polymers other than PEG (like PAA and PVP in the case described), when made amphiphilic by modification at one terminus with long-chain fatty acyl, can incorporate into the liposome surface and make liposome long-circulating. The protection effects observed depend on both the length of hydrophobic 'anchor', and the length of hydrophilic polymer chain, and might be interpreted in terms of the balance between the energy of hydrophobic anchor interaction with membrane core and the energy of polymer chain motion in the water solution.

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