PREPARATION OF TEMPERATURE-SENSITIVE LIPOSOMES FOR DELIVERY OF ANTICANCER DRUGS BY USE OF THERMOSENSITIVE AMPHIPHILIC BLOCK COPOLYMER

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Introduction

Liposomes are highly attractive materials for drug delivery because of their biocompatibility, ability to encapsulate both hydrophilic and hydrophobic drugs, and their size controllability. Therefore, studies to modify their functions have been conducted to increase their usefulness. While various functional liposomes have been developed, temperature-sensitive liposomes have received much attention. Temperature-sensitive liposomes have been produced by modifying liposomes with thermosensitive polymers, such as copolymers of N-isopropylacylamide with the lower critical solution temperature (LCST) around physiological temperature (1-4).

Recently, we have shown that surface modification with block copolymers of (2-ethoxy)ethoxy ethyl vinyl ether (EOEOVE) and octadecyl vinyl ether (ODVE) gives liposomes temperature-sensitive properties, such as contents release and surface hydrophobicity-hydrophilicity (5). In this study, liposomes that release anticancer drug, doxorubicin (DOX), a few degree above physiological temperature were designed by use of these block copolymers. The poly(EOEOVE) block behaves as a thermosensitive moiety with the lower critical solution temperature of about 40°C, whereas the poly(ODVE) moiety acts as anchor for the copolymer fixation onto liposomal membranes. The performance of the copolymer-modified liposomes as tumor-specific delivery system for anticancer drug was investigated.

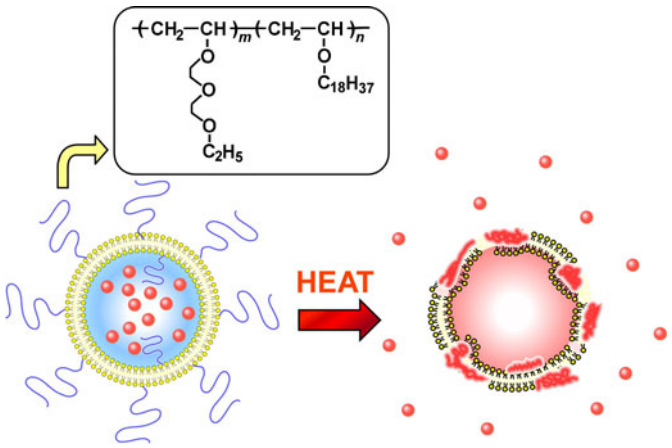

Figure 1. Design of temperature-sensitive liposomes using poly(2-ethoxy)ethoxy ethyl vinyl ether-*block*-octadecyl vinyl ether).

Experimental Part

Block copolymerization of EOEOVE and ODVE was carried out as follows (Figure 2). EOEOVE was first polymerized in the presence of $CH_3COOC_2H_5$, 1-isobutylethyl acetate and $Et_{1.5}AlCl_{1.5}$ in toluene. After 2.5 h, the second monomer, ODVE was added to the reaction mixture at a given conversion of EOEOVE. After 2 h, ODVE was consumed quantitatively (total 4.5 h). When the polymerization was finished, it was quenched with methanol containing a small amount of aqueous ammonia. The quenched reaction mixture was diluted with dichloromethane and then sequentially washed with dilute hydrochloric acid and with water to remove the initiator residues. The product copolymer was recovered from the organic layer by evaporation of the solvent under reduced pressure

and vacuum-dried overnight.

The DOX-loaded egg yolk phosphatidylcholine (EYPC)-cholesterol liposomes modified with the copolymer were prepared by hydration of a mixture of the lipids and copolymer and subsequent loading of DOX into the liposomes by the pH-gradient method. A mixture of EYPC and the copolymer was dissolved in chloroform, and the solvent was removed by evaporation. The obtained thin lipid/copolymer-mixed membrane was dispersed in an aqueous ammonium sulfate solution (pH 5). The obtained liposome suspension was extruded through a polycarbonate membrane with a pore diameter of 100 nm in an ice-cooled water bath. The liposomes were applied to a Sepharose 4B column at 4°C using a Hepes buffered solution (pH 7.4). An aqueous DOX solution was added to the liposomes suspension and incubated for 1h at 30°C and then, the mixed solution was applied to a Sepharose 4B column to afford the DOX-loaded liposomes.

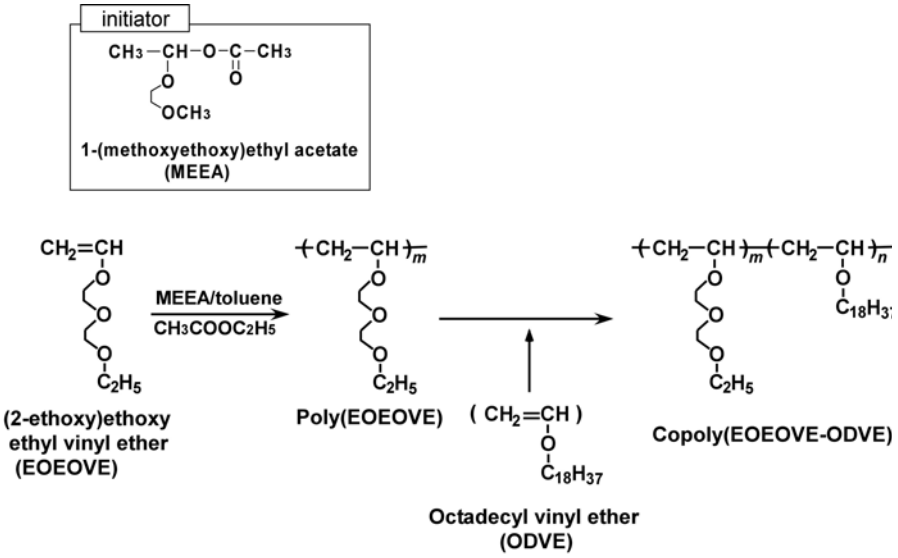

Figure 2. Synthetic route for poly(2-ethoxy)ethoxy ethyl vinyl ether-*block*-octadecyl vinyl ether).

Results and discussion

The composition of the block copolymer of EOEOVE and ODVE prepared in this study was estimated to be 93/4 (EOEOVE/ODVE, mol/mol) using ^{1}H NMR. The number-average and weight-average molecular weights of the copolymer were evaluated to be 15900 and 16900, respectively.

Conformational transition of the copolymer was detected using differential scanning calorimetry. The copolymer was found to undergo a coil-globule transition at ca. 40ºC in the presence of a membrane of EYPC.

Figure 3 represents release of DOX from the DOX-loaded liposomes modified with varying amounts of the copolymer. Although the liposome without the copolymer hardly released the content between 20 and 50ºC, the liposomes having the copolymer exhibited temperature-sensitive release behavior. These copolymer-modified liposomes encapsulating DOX released little DOX below 40ºC, whereas the release was strongly enhanced above 40ºC. Because that temperature corresponds to the conformational transition temperature of the

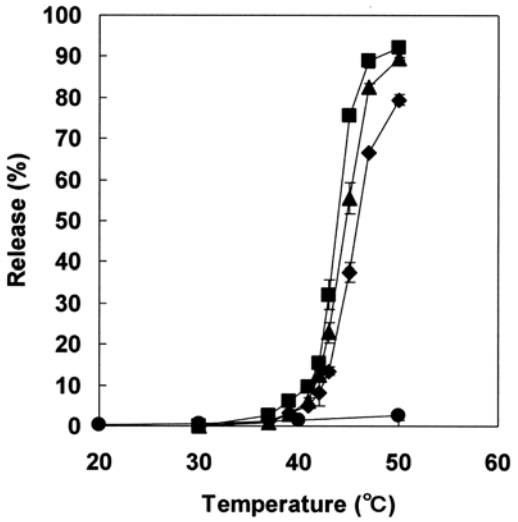

Figure 3. Percent release of DOX from EYPC/cholesterol/copolymer modified liposomes with copolymer contents (mol%) of 0 (circles), 0.3 (diamonds), 1.0 (triangles) and 2.0 (squares). After 3 min incubation.

copolymer, it is likely that the dehydrated copolymer chains attached to the liposome surface destabilized the liposome membrane, inducing the content release. In comparison among the liposomes modified with varying copolymer contents, it is apparent that the DOX release was enhanced more strongly above 40ºC with increasing copolymer content.

Similarly, we examined the effect of surface modification with the copolymer on the DOX release behavior of EYPC/cholesterol liposomes containing poly(ethylene glycol) (PEG)-attached lipid. We found that the copolymer-modified liposomes with PEG grafts strongly enhanced the release of DOX above 40ºC: more than 80% of DOX was released from the liposomes within 1 min at 45°C. This result indicates that the surface modification with the copolymer also gave the temperature-sensitive content release property to the PEG-modified liposomes, which exhibit long circulating property.

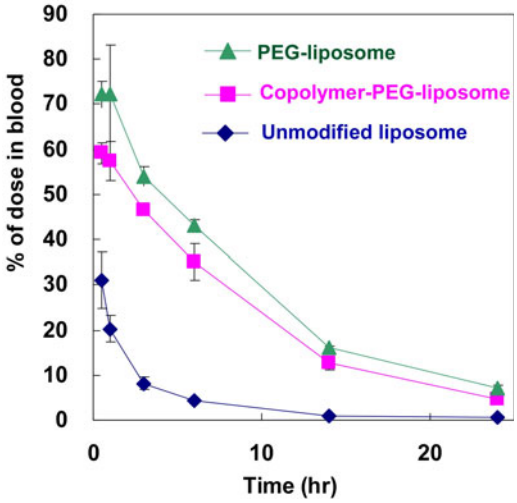

Figure 4. Clearance of various liposomes from the blood circulation in mice as a function of time after intravenous injection.

Next, distribution of the copolymer-modified liposomes in the bodyof mice was examined by using radioisotope-labeled liposomes (Figure 4). The liposomes were injected into tail vein of mice and their distribution in the body was followed. Although the copolymer-modified liposomes exhibited slightly longer blood circulation time than bare liposomes, these liposomes were quite quickly taken up by liver. However, the liposomes modified with PEG-lipid and the copolymer showed a long circulation property similar to that of PEG-modified liposomes.

Finally, antitumor activity of the DOX-loaded liposomes modified with the copolymer and PEG was investigated (Figure 5). The copolymer-PEG-modified liposomes containing DOX were injected into tail vein of mice bearing tumor. Tumor growth was slightly suppressed by the DOX-loaded liposomes when the tumor was not heated. However, when the tumor was heated at 45°C for 10 min, their efficacy for the suppression of tumor growth increased dramatically. The same heat treatment hardly affected tumor growth when temperature-insensitive PEG-modified, DOX-loaded liposomes were injected. Therefore, it is likely that the copolymer-PEG-modified, DOX-loaded liposomes accumulated at the tumor site efficiently and released the anticancer drug responding to heat application, resulting in the strong suppression of its growth.

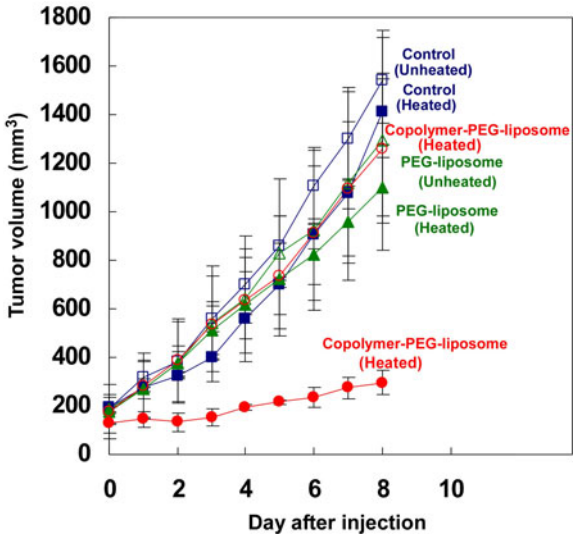

Figure 5. Time course of tumor growth after injection of Hepes saline, DOX-loaded PEG-modified liposome, and DOX-loaded copolymer-PEG-modified liposome with (solid symbols) of without (open symbols) heat application.

The results obtained in this study demonstrate that the liposomes modified with the copolymer and PEG have potential usefulness for delivery of antitumor drugs to the target tumor.

References

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