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REMARKABLY ENHANCED INHIBITORY EFFECTS OF HYBRID LIPOSOMES ON THE GROWTH OF SPECIFIC TUMOR CELLS

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Abstract : The highly specific inhibitory effects of the hybrid liposomes composed of 90 mol% L-α-dimyristoylphosphatidylglycerol and 10 mol% polyoxyethylene (10) dodecyl ether on the growth of lung adenocarcinoma and stomach tumor cells *in vitro* were obtained for the first time.

Liposomes have recently attracted attention in connection with reducing the toxicity of antitumor drugs.[1] We have recently produced new-type hybrid liposomes composed of vesicular and micellar molecules. The physical properties of these liposomes, such as size, membrane fluidity, phase transition temperature, and hydrophobicity can be controlled by changing the composition of hybrid liposomes.[2] In the course of our study, remarkably high inhibitory effects of hybrid liposomes on tumor growth *in vitro* without drugs have been obtained.[3~7] The fluorescence micrograph taken using fluorescein isothiocyanate as a fluorescence probe showed clearly that the hybrid liposomes might be fused with the tumor cell membrane. We represent a hypothetic mechanism that hybrid liposomes should be promote the fusion with tumor cells and the microenvironment of tumor cell membranes should be changed. As a result, the growth signal should be blocked after the conformation of receptors should be changed. At any rate, it was noteworthy that the induction of apoptosis by the hybrid liposomes in leukimia cells was verified on the basis of DNA agarose gel electrophoresis, flow cytometer and microphysiometer.[7] No toxicity of the hybrid liposomes was observed in normal cells *in vitro* and in normal rats *in vivo* without any side effects.[8, 9] No incorporation into normal human blood vessel endothelial cells was obserbed using fluorescence micrograph. Significantly prolonged survival was obtained using mice of carcinoma *in vivo*.[10]

In this study, the inhibitory effects of hybrid liposomes composed of lipids having a variety of head groups (zwitter ionic L-α-dimyristoylphosphatidylcholine (DMPC), anionic L-α-dimyristoylphosphatidylglycerol (DMPG), and cationic 1,2-dimyristoyl-3-trimethylammonium propane (DMTAP)) and polyoxyethylene (10) dodecyl ether ($C_{12}(EO)_{10}$) on the growth of tumor cells (human lung adenocarcinoma (RERF-LC-OK), human hepatoma(Hep-G_2), human stomach tumor (GT3TKB)) *in vitro* were examined.

Firstly, we examined the physical property of the hybrid liposomes composed of lipids and $C_{12}(EO)_{10}$

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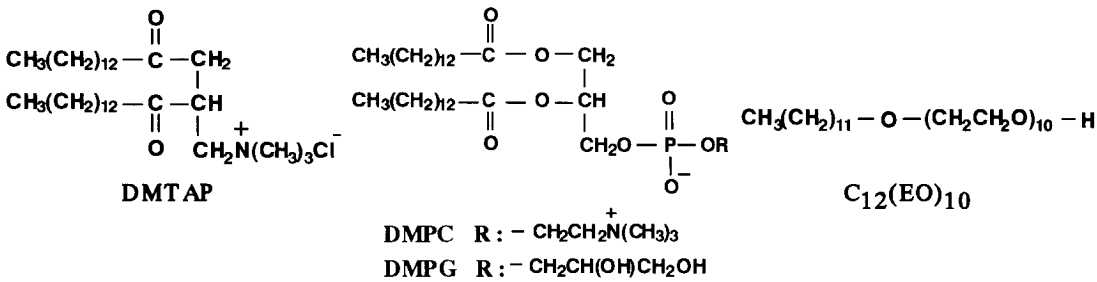

micellar molecules. Time course of changes in the hydrodynamic diameter of hybrid liposomes estimated at 37 ℃ is shown in Figure 1.[11] The hybrid liposomes were prepared by dissolving both lipids and $C_{12}(EO)_{10}$ in phosphate-buffered saline with sonication (BRANSONIC Model B2210 apparatus, 90 W) at 45 °C for 5 min. Interestingly, the hybrid liposomes were found to be stable for 3-5 weeks, having a single distribution of hydrodynamic diameter. On the other hand, unclear solutions were obtained in the case of lipids only. This stability of these hybrid liposomes should be advantageous for the clinical application in future.

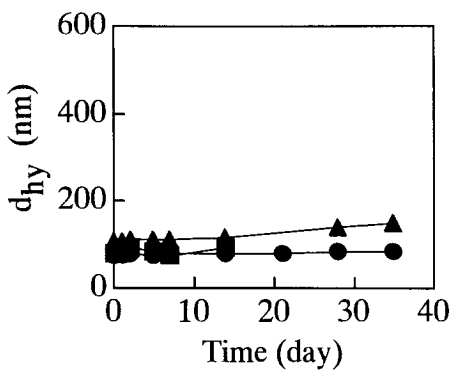

Fig.1 Time Course of d_{hy} Change for Hybrid Liposomes Composed of 90mol% Lipid and 10mol%$C_{12}(EO)_{10}$ (● : DMPC /10mol%$C_{12}(EO)_{10}$, ▲ : DMPG/10mol%$C_{12}(EO)_{10}$, ■ : DMTAP/10mol%$C_{12}(EO)_{10}$).

Secondly, inhibitory effects of DMTAP/10 mol% $C_{12}(EO)_{10}$, DMPG/ 10 mol% $C_{12}(EO)_{10}$ and DMPC/10 mol% $C_{12}(EO)_{10}$ on the growth of tumor cells (human lung adenocarcinoma (RERF-LC-OK), human hepatoma (Hep-G_2), human stomach tumor (GT3TKB)) *in vitro* were examined on the basis of WST-1 method.[12] The cells were cultured for 2 days in a 5% CO_2 incubator at 37℃ after adding the hybrid liposomes. The inhibitory effects of hybrid liposomes on the growth of tumor cells were evaluated by $A_{mean}/A_{control}$, where A_{mean} and $A_{control}$ denote the absorbance of water-soluble formazan in the presence and absence of the hybrid liposomes, respectively. The results are shown in Figures 2, 3 and 4. The inhibitory effects of the hybrid liposomes composed of cationic DMTAP on the growth of all the tumor cells employed are the same as those of the DMTAP liposomes. This result may be caused by the toxicity of cationic DMTAP that was found in animal experiments *in vivo*.[13] It is noteworthy that the hybrid liposomes of DMPC/10 mol% $C_{12}(EO)_{10}$ and DMPG/10 mol% $C_{12}(EO)_{10}$ were fairly more effective for inhibiting the growth of all tumor cells employed in this study as compared with liposomes (DMPC and DMPG) or micelles [$C_{12}(EO)_{10}$]. Significantly prolonged survival (183%) was obtained by using mice model of carcinoma treated with the hybrid liposomes of DMPC/10 mol%

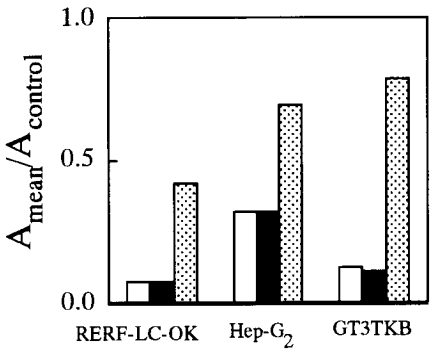

Fig. 2 Inhibitory Effect of Hybrid Liposomes Composed of 90mol% DMTAP and 10mol% $C_{12}(EO)_{10}$ on Growth of Various Tumor Cells.
(□ : DMTAP, ⊡: $C_{12}(EO)_{10}$, ■ : DMTAP / 10mol % $C_{12}(EO)_{10}$). [DMTAP] = 3.00×10^{-4} M, [$C_{12}(EO)_{10}$]=3.33×10^{-5} M

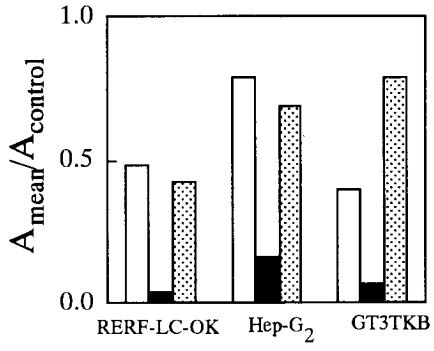

Fig. 3 Inhibitory Effect of Hybrid Liposomes Composed of 90mol% DMPG and 10mol% $C_{12}(EO)_{10}$ on Growth of Various Tumor Cells.
(□ : DMPG, ⊡: $C_{12}(EO)_{10}$, ■ : DMPG / 10mol % $C_{12}(EO)_{10}$). [DMPG] = 3.00×10^{-4} M, [$C_{12}(EO)_{10}$]=3.33×10^{-5} M

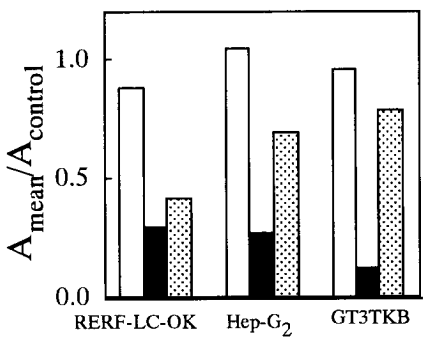

Fig. 4 Inhibitory Effect of Hybrid Liposomes Composed of 90mol% DMPC and 10mol% $C_{12}(EO)_{10}$ on Growth of Various Tumor Cells.
(□ : DMPC, ⊡: $C_{12}(EO)_{10}$, ■ : DMPC / 10mol % $C_{12}(EO)_{10}$). [DMPC] = 3.00×10^{-4} M, [$C_{12}(EO)_{10}$]=3.33×10^{-5} M

$C_{12}(EO)_{10}$.[10] It is attractive that the specific inhibitory effect of the hybrid liposomes of DMPG/10 mol% $C_{12}(EO)_{10}$ on the growth of RERF-LC-OK and GT3TKB cells was obtained. It has been reported that in the DMPG liposomes, in contrast to the DMPC liposomes, the hydration can proceed almost limitlessly,[14] and the extent of surface hydration[15] has been considered as one of the factors affecting the anticancer activity.

In conclusion, the hybrid liposomes of DMPG/10 mol% $C_{12}(EO)_{10}$ were found to be highly effective for inhibiting the growth of lung adenocarcinoma and stomach tumor cells for the first time.

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