SPECIFIC HYBRID LIPOSOMES COMPOSED OF PHOSPHATIDYLCHOLINE AND POLYOXYETHYLENEALKYL ETHER WITH MARKEDLY ENHANCED INHIBITORY EFFECTS ON THE GROWTH OF TUMOR CELLS IN VITRO

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The hybrid liposomes (90mol% DMPC/10mol% $C_{12}(EO)_8$ and 90mol% DMPC/10mol% $C_{12}(EO)_{12}$) have a highly inhibitory action against the growth of tumor cells. The uniform and stable structure of the hybrid liposomes was revealed on the basis of electron microscopy and dynamic light scattering measurements.

KEY WORDS liposome; in vitro antitumor activity; polyoxyethylenealkyl ether

It is well known that liposomes are used as drug carriers; examples are antitumor agents, hormones, and immunomodulation.^{1,2)} In particular, polyoxyethyleneglycol (PEG)-phosphatidylcholine (PC) liposomes have been found to be effective for prolonging blood circulation.^{3,4)} On the other hand, we have recently produced specific hybrid liposomes composed of vesicular and micellar molecules, whose physical properties (sizes, membrane fluidity, phase transition temperature, hydrophobicity) could be controlled by changing their composition.⁵⁻⁶⁾

In the course of our study on the liposomes, inhibitory effects of hybrid liposomes including flavonoids⁷⁾ or antitumor drugs⁸⁾ on hybridoma growth in vitro have been observed, though a lower survival ratio was obtained in the presence of synthetic quaternary ammonium surfactants or antitumor drugs. However, a remarkably high inhibitory effect of liposomes without drugs has not been reported so far.

In this study, we report for the first time on the uniform and stable hybrid liposomes composed of dimyristoylphosphatidylcholine (DMPC) and polyoxyethylenealkyl ether ($C_m(EO)_n$) having inhibitory action toward the growth of tumor cells in vitro.

Firstly, we examined the morphology of the hybrid liposomes⁹⁾ composed of 90mol% DMPC and 10mol% $C_{12}(EO)_{12}$ on the basis of electron microscopy¹⁰⁾ and dynamic light scattering (dls)¹¹⁾ data. The electron micrograph of the hybrid liposomes (Fig.1) shows the presence of spherical vesicles. Interestingly, a clear solution of the hybrid liposomes having a hydrodynamic diameter (d_{hy}) of 130 - 140 nm with a single and narrow distribution could be kept over 30 days, as shown in Fig.2. These

Fig. 1. Electron Micrograph of Hybrid Liposomes Composed of DMPC and $C_{12}(EO)_{12}$

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Fig.2. Distribution of d_{hy} and Time Course of d_{hy} Change for Hybrid Liposomes Composed of DMPC and $C_{12}(EO)_{12}$

results indicate that the uniform and stable hybrid liposomes composed of 90mol% DMPC and 10mol% $C_{12}(EO)_{12}$ could be obtained.

Secondly, the inhibitory effects of the hybrid liposomes composed of DMPC and $C_m(EO)_n$ on the growth of human lymphoma-human B lymphocyte hybridoma cells¹²⁾ (HF cells) were examined. The cells were cultured for 4 days at 37°C in a humidified atmosphere of 5% CO_2 / 95% air after adding the hybrid liposomes. The inhibitory effect of

Table.1. Inhibitory Effect of Hybrid Liposomes Composed of DMPC and $C_m(EO)_n$ on the Growth of Hybridoma Cells

Sample	Inhibitory Effect (%)	
DMPC	9	
DMPC/10mol% $C_{10}(EO)_4$	67	(24)
DMPC/10mol% $C_{10}(EO)_8$	61	(32)
DMPC/10mol% $C_{12}(EO)_4$	50	(6)
DMPC/10mol% $C_{12}(EO)_7$	86	(36)
DMPC/10mol% $C_{12}(EO)_8$	94	(65)
DMPC/10mol% $C_{12}(EO)_{12}$	91	(60)
DMPC/10mol% $C_{12}(EO)_{23}$	88	(29)
DMPC/10mol% $C_{14}(EO)_4$	62	(4)
DMPC/10mol% $C_{14}(EO)_6$	95	(22)
DMPC/10mol% $C_{14}(EO)_7$	96	(54)
DMPC/10mol% $C_{14}(EO)_8$	95	(70)

[DMPC] = $1.5 \times 10^{-4} M$, [$C_m(EO)_n$] = $1.7 \times 10^{-5} M$. Initial Cell Number : $8.9 \sim 11.9 \times 10^4 \text{ ml}^{-1}$. Values in the parentheses are those of $C_m(EO)_n$ micelles.

The inhibitory effects have maximum errors of $\pm 5\%$.

hybrid liposomes on the growth of tumor cells was evaluated on the basis of $100(N_a - N_p)/N_a$, where N_a and N_p denote the live cell number in the absence and presence of the hybrid liposomes, respectively. The results are summarized in Table 1. The noteworthy aspects are as follows: (a) The inhibitory effects of the hybrid liposomes composed of DMPC and $C_m(EO)_n$ ($m = 10, n = 4$ and 8 ; $m = 12, n = 4$; $m = 14, n = 4$) are moderately enhanced as compared with those of liposomes of DMPC or micelles of $C_m(EO)_n$. (b) The inhibitory effects of DMPC/ $C_{12}(EO)_7$ and DMPC/ $C_{12}(EO)_{23}$ hybrid liposomes were remarkably attractive. (c) The almost completely inhibitory effects were attained by employing the hybrid liposomes of DMPC/ $C_{12}(EO)_n$ ($n = 8, 12$) and $C_{14}(EO)_n$ ($n = 6, 7, 8$). These results suggest that the hydrophilic-hydrophobic balance in $C_m(EO)_n$ micelles should be of importance for the enhancement of inhibitory effect on the growth of tumor cells. The inhibitory effects of the hybrid liposomes composed of DMPC and $C_{12}(EO)_n$ ($n = 8$ and 12) on the growth of human T lymphoma and glioma cell lines were also obtained.

It is also of importance that high viability ($70 \sim 100\%$)¹³⁾ was attained in all hybrid liposomes employed in this study. Furthermore, no inhibition toward normal human tunica mucosa esophage cells in vitro¹⁴⁾ and no toxicity to normal rats in vivo¹⁵⁾ were observed.

In conclusion, it is worthy of note that almost perfect inhibitory action against the growth of tumor cells was attained by using the clear solutions of 90mol% DMPC and 10mol% $C_{12}(EO)_n$ ($n = 8, 12$) and $C_{14}(EO)_n$ ($n = 6, 7, 8$) hybrid liposomes without drugs for the first time.

ACKNOWLEDGEMENT We thank Prof. Tetsuro Yamamoto of Kumamoto University Medical School and Dr. H. Inoue of Panapharm Laboratories Co., Ltd. for providing experimental data relating to normal human cells and normal rats, respectively. This work was supported in part by a Grant-in-Aid for Science Research from the Ministry of Education, Science, and Culture of Japan (No. 07808101).

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- 10) The electron micrograph obtained by means of cryotransfer technique was taken on a JOEL JEM-2000FX II electron microscope.
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(Received July 3, 1995; accepted August 21, 1995)