Chemotherapy with Hybrid Liposomes for Melanomatosis

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The inhibitory effects of the hybrid liposomes on the growth of B16 melanoma cells in vitro and in vivo were examined. The 50% inhibitory concentration of the hybrid liposomes composed of 90 mol% dimyristoylphosphatidylcholine (DMPC) and 10 mol% polyoxyethylatededecyl ether (C12(EO)n) was one-twelfth of that of DMPC liposomes. It was noteworthy that for the first time significantly prolonged survival was obtained using a nude mouse model of carcinoma after the administration of the hybrid liposomes of 90 mol% DMPC/10 mol% C12(EO)n (n=10 or 23) without antitumor drugs.

Keywords liposome; chemotherapy; antitumor; melanoma

Liposomes are closed vesicles that are mainly composed of phospholipids, the constituent of biological membranes, and are used in various ways as safe drug carriers. However, many technical problems remain be solved. The preparation of liposomes is a rather complicated process, and it is possible that organic solvents may contaminate the liposomes prepared. The stability of liposomes is an essential prerequisite for their therapeutic application.

We have previously developed a new medical material of hybrid liposomes. The hybrid liposomes can be prepared simply by sonication in buffer solution containing phospholipids and micellar surfactants; they are free from any contamination from organic solvents and stable for a longer period. Furthermore, it is possible to regulate the shape and size, temperature of phase transition, hydrophobicity, and fluidity by changing the components and their relative proportions. We have also demonstrated that the hybrid liposomes are effective in inhibiting the growth of B cell lymphoma or leukemia cells in vitro. No toxicity of the hybrid liposomes was observed in normal cells in vitro or in normal rats in vivo with no side effects. Hybrid liposomes containing lipid-soluble antitumor agents were further found to prolong survival significantly using an in vivo mouse model of glioma.

In the present study, the antitumor effects of hybrid liposomes composed of l-α-dialkylphosphatidylcholine (DMPC or DPPC) and polyoxyethylatededecyl ether (C12(EO)n, n=10 or 23) on the growth of mouse melanoma cells (B16 melanoma) in vitro and in vivo were examined.

First, we examined the 50% inhibitory concentration (IC50) of the hybrid liposomes on the growth of B16 melanoma cells in vitro. The cells were cultured for 2 d in a 5% CO2 incubator at 37 °C after adding the hybrid liposomes. The IC50 of the hybrid liposomes on the growth of B16 melanoma cells was determined by WST-1 assay. The hybrid liposomes were prepared by dissolving both phospholipids and C12(EO)n in phosphate-buffered saline with sonication (BRANSONIC Model B2210 apparatus, 90 W) at 45 °C for 5 min in an atmosphere of nitrogen and filtered in a sterile manner through a 0.45 μm Millipore filter. The results are summarized in Fig. 1. The noteworthy aspects are as follows: 1) The IC50 of the hybrid liposomes of DMPC/C12(EO)n and DMPC/C12(EO)b were one-twelfth and one-fourth of that of DMPC liposomes, respectively. 2) The IC50 of the hybrid liposomes of DPPC/C12(EO)n was one-third of that of DPPC liposomes.

How do these hybrid liposomes inhibit the growth of tumor cells? A fluorescence micrograph taken using fluorescein isothiocyanate as a fluorescence probe showed that the hybrid liposomes may fuse with the tumor cell membrane. We propose a hypothetical mechanism whereby hybrid liposomes promote fusion with tumor cells and the microenvironment of tumor cell membranes therefore changes. As a result, the growth signal is blocked after the conformation of receptors is altered. When the apoptotic DNA rate for B16 melanoma cells treated with hybrid liposomes of DMPC/C12(EO)n was observed using a flow cytometer, induction of apoptosis by the hybrid liposomes in B16 melanoma cells was verified.

Second, we examined the inhibitory effects of hybrid liposomes in mice intraperitoneally inoculated with B16 melanoma cells in vivo. These cells were originally from skin tumors but can be taken as a model of lung cancer, since melanoma frequently metastasizes to the lymph nodes and lungs. Screening for therapeutic effects is generally carried out in mice or rats bearing tumor cells. In the primary screening, tumor cells are introduced into the peritoneal cavity, and the test agent is administered intraperitoneally. Animals were randomly grouped on the basis of body weight on the day of tumor cell inoculation using the stratified randomization method. B16 melanoma cells (5×106 cells) were inoculated intraperitoneally. Hybrid liposomes were administered 13 times starting from 1 h after the inoculation every day. The median life span was calculated using the equation: (median survival days after treatment)/(median survival days of control group)×100. The results are shown in Fig. 2. The therapeutic effects of DMPC or DPPC liposomes were not examined because clear stock solutions of DMPC or DPPC were not maintained for two days. The median life span was 103% and 115% in the C12(EO)n-treated group and

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C_{12}(EO)_{33}-treated group, respectively. It is noteworthy that significantly prolonged survival (162—170%) was obtained in mice treated with both the hybrid liposomes of DMPC/10 mol% C_{12}(EO)_{10} and DMPC/10 mol% C_{12}(EO)_{23}. On the other hand, DPPC/10 mol% C_{12}(EO)_{23} hybrid liposomes had no life-prolonging effects. The difference in the antitumor effect of *in vitro* and *in vivo* DPPC/10 mol% C_{12}(EO)_{23} hybrid liposomes is currently under investigation.

In conclusion, significantly prolonged survival was obtained for the first time using a mouse model of carcinoma after the administration of hybrid liposomes without antitumor drugs.

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REFERENCES AND NOTES