

Highly Specific Inhibitory Effect of Three-Component Hybrid Liposomes Including Sugar Surfactants on the Growth of Glioma Cells

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Abstract—Three-component hybrid liposomes composed of L- α -dimyristoylphosphatidylcholine, micellar surfactant (Tween 20), and β -D-fructofuranosyl- α -D-glucopyranoside monododecanoate were found to be highly effective for inhibiting the growth of glioma cells without any drug. © 2000 Elsevier Science Ltd. All rights reserved.

Liposomes are closed vesicles composed of a bimolecular membrane resembling the biological membrane of a cell composed primarily of phospholipids.¹ Recently, a new type of hybrid liposome composed of vesicular and micellar molecules has been produced.² The hybrid liposomes can be prepared simply by sonication in a buffer solution containing phospholipids and micellar surfactants. They are free from any contamination from organic solvent and stable for more than 1 month. It has been possible to regulate the shape and size, phase transition temperature, hydrophobicity, and fluidity by changing the components and their proportions. These hybrid liposomes have been effective for inhibiting the growth of lymphoma,^{3–5} leukemia,⁶ melanoma⁷ and lung adenocarcinoma⁸ cells in vitro. Significantly prolonged survival was also obtained in vivo in mice with carcinomas.^{7,9} Interestingly, hybrid liposomes showed no toxicity in normal human cells, and no side effects or toxicity in vivo in rats.¹⁰

It is well known that sugars play important roles in recognizing molecules on the cell membranes through receptors including lectins for some kinds of sugars.¹¹ So, in this study, we report for the first time on the inhibition of tumor cell growth by three-component hybrid liposomes composed of L- α -dimyristoylphosphatidylcholine (DMPC), micellar surfactant (Tween 20), and β -D-fructofuranosyl- α -D-glucopyranoside monoacylate (SucC_n; $n = 10, 12$) having a different acyl chain.

Firstly, we examined the morphology of the three-component hybrid liposomes composed of DMPC, Tween 20 and SucC₁₂ on the basis of electron microscopy.¹² The electron micrographs (Fig. 1) of the two-component hybrid liposomes (A) and three-component hybrid liposomes (B) show the presence of spherical vesicles with a diameter of 100–200 nm.

Secondly, the inhibitory effect of the three-component hybrid liposomes composed of DMPC, Tween 20 and SucC_n ($n = 10, 12$) on the growth of various tumor cells (human glioma (U251), human hepatoma (Hep-G2), human lung adenocarcinoma (RERF-LC-OK)) was examined. The cells were cultured in a 5% CO₂ incubator at 37 °C. The inhibitory effect of hybrid liposomes on the growth of the cells was evaluated by calculating $A_{\text{mean}}/A_{\text{control}}$, where A_{mean} and A_{control} denote the absorbance of water-soluble formazan in the presence and absence of the hybrid liposomes, respectively, on the basis of the WST-1 method.¹³ The results are shown in Fig. 2. No significant inhibitory effect of any individual component (DMPC, Tween 20, SucC_n) or the two-component hybrid liposomes on the growth of all the tumor cells examined was obtained. It is noteworthy that the three-component hybrid liposomes composed of DMPC, Tween 20 and SucC₁₂ were remarkably effective for inhibiting the growth of glioma (U251) cells. The mechanism of antitumor effect of the three-component hybrid liposomes including sugar micelles (SucC₁₂) may involve adhesion molecules such as lectins which may play an important role in inhibiting the growth of tumor cells. It is known that the affinity of

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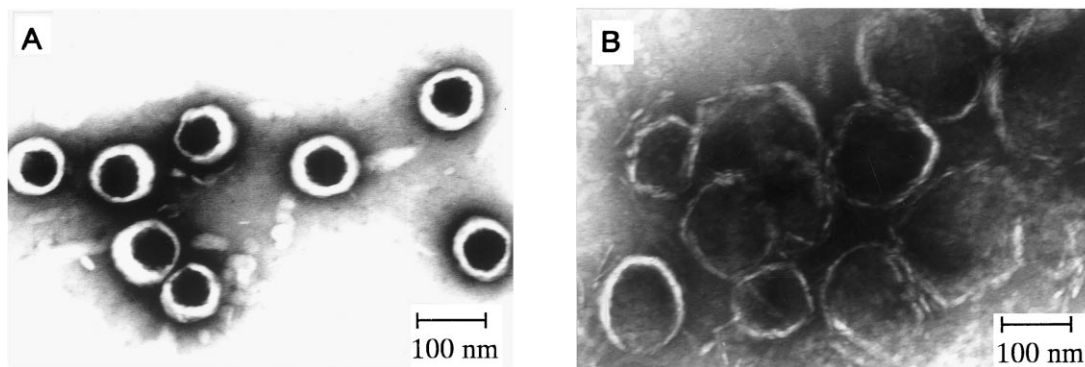
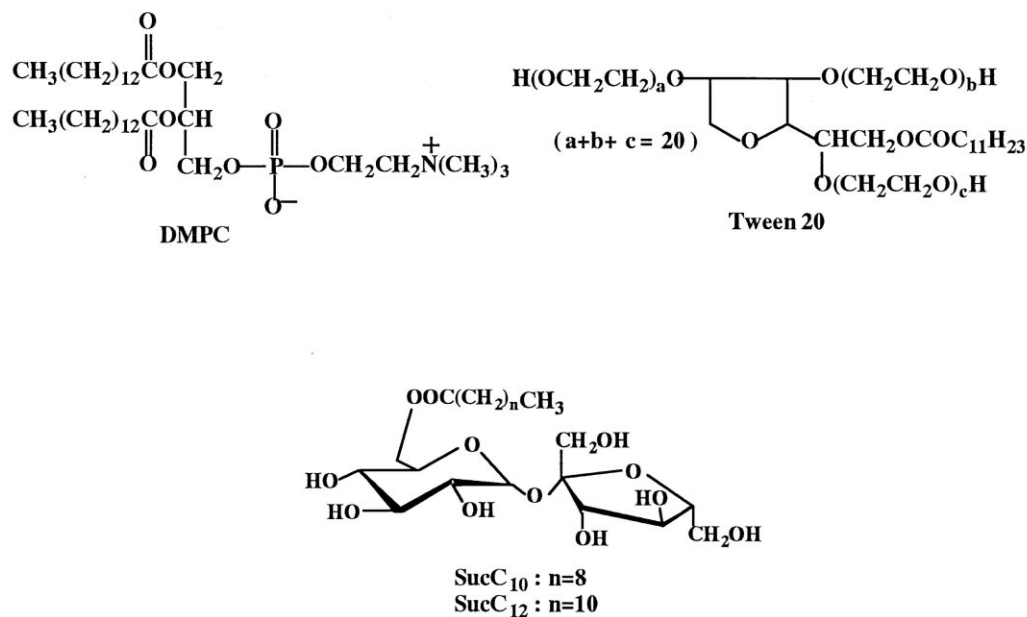


Figure 1. Electron micrographs of (A) the two-component hybrid liposomes composed of DMPC and Tween 20, and (B) the three-component hybrid liposomes composed of DMPC, Tween 20 and SucC₁₂.

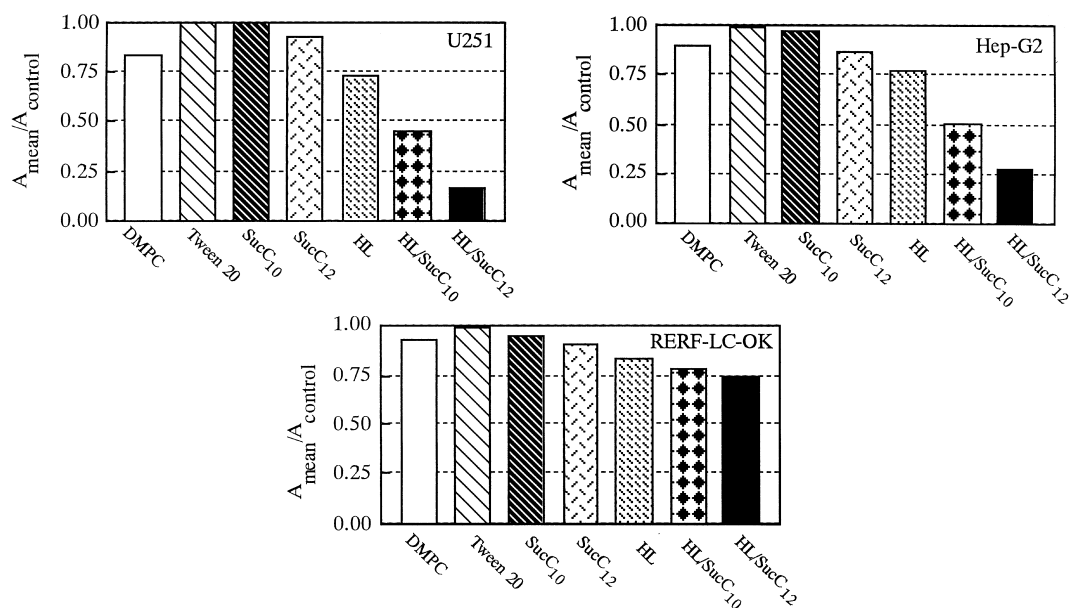


Figure 2. Inhibitory effect of three-component hybrid liposomes composed of DMPC, Tween 20 and SucC₁₂ on the growth of various tumor cells in vitro (HL: hybrid liposomes composed of DMPC and Tween 20).

sugar chains for their recognition molecules, such as lectins, is not particularly strong. However, it is generally accepted that the expression of these lectins increases in tumor cells, and that this increase plays an important role in the mechanism of metastasis of tumor cells.¹⁴

The membrane fluidity was determined using 1,6-diphenyl-1,3,5-hexatriene (DPH) and 1-(4-trimethyl ammonium phenyl)-6-phenyl-1,3,5-hexatriene iodide (tma-DPH) as fluorescence probes. The *p* value of DPH or tma-DPH was almost the same in the experiments using the two- and three-component hybrid liposomes. The thickness of the fixed aqueous layer of the three-component hybrid liposomes composed of DMPC, Tween 20 and SucC₁₂ (4.9 nm) was twice that of the two-component hybrid liposomes composed of DMPC and Tween 20 (2.5 nm). This result suggests that the inhibitory effect of the three-component hybrid liposomes might be related to hydration of tumor cell membrane. Furthermore, apoptosis induction by the three-component hybrid liposomes composed of DMPC, Tween 20 and SucC₁₂ was found in U251 cells on the basis of flow cytometry, although some cells were shifted to G2/M.

In conclusion, these three-component hybrid liposomes composed of DMPC, Tween 20 and SucC₁₂ showed a highly specific inhibitory effect on the growth of glioma cells in vitro without any drug.

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