Remarkably Enhanced Inhibitory Effects of Three-Component Hybrid Liposomes Including Sugar Surfactants on the Growth of Lung Carcinoma Cells

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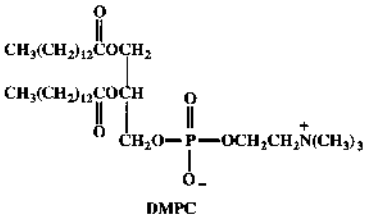
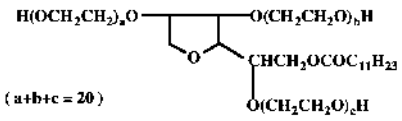
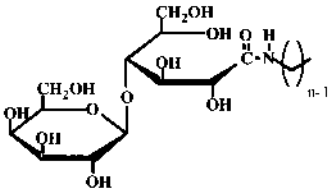
Sugar parts play important roles in recognizing molecules on the cell membranes. We successfully produced sugar-type micellar surfactants, lactonoalkylamide ($LacC_n$), for the first time. Spherical vesicles, three-component hybrid liposomes, were obtained after the sonication of the mixture of L-α-dimyristoylphosphatidylcholine (DMPC), Tween 20 and $LacC_n$ (DMPC : Tween 20 : $LacC_n$ = 65 : 7 : 28). It is noteworthy that high inhibitory effects of the three-component hybrid liposomes composed of DMPC, Tween 20, and $LacC_n$ (DMPC : Tween 20 : $LacC_n$ = 65 : 7 : 28) on the growth of glioma (U251) and lung adenocarcinoma (RERF-LC-OK) cells were attained *in vitro* without any drug, although no significant inhibitory effects of any individual component (DMPC, Tween 20, $LacC_n$) or the two-component hybrid liposomes of DMPC and Tween 20 on the growth of tumor cells examined were obtained.

Key words liposome; sugar surfactant; antitumor







Liposomes have attracted attention in connection with reducing the toxicity of antitumor drugs.[1] New-type hybrid liposomes composed of vesicular and micellar molecules have been produced.[2] The hybrid liposomes can be prepared simply by sonication in a buffer solution without organic solvent and they remain stable for more than one month. It has been possible to regulate the shape and size, phase transition temperature, hydrophobicity, and fluidity by changing the composition. These hybrid liposomes have been effective for inhibiting the growth of tumor cells *in vitro*.[3—7] Significantly prolonged survival has been also obtained *in vivo* using mice model of carcinoma after treatment with hybrid liposomes.[8—10] The hybrid liposomes showed no toxicity *in vivo* using normal rats.[10,11]

It is well known that sugar parts play important roles in recognizing molecules on the cell membranes through receptors including lectins.[12] Recently, preparation and characterization of glyco-liposomes has been reported.[13,14] Lactose is a disaccharide composed of D-galactose to play an important role in molecular recognition *in vivo*.[15] So, in this study, we report on the three-component hybrid liposomes composed of L-α-dimyristoylphosphatidylcholine (DMPC), micellar surfactant (Tween 20), and lactonoalkylamide ($LacC_n$; n=12, 14, 16) related to inhibition of the growth of tumor cells.

Firstly, we prepared $LacC_n$ from alkylamine and lactonolactone,[16] which was obtained by dehydration of lactobionic acid (Fig. 1). Methanol solution of lactobionic acid was evaporated in vacuum. During the evaporation, an appropriate amount of dried methanol was added to dissolve and remove the lactobionic acid. Dried methanol solution of lactonolactone was added to dried methanol solution of alkylamine and the mixture was refluxed for 12 h. The product was separated with a filter paper, washed with dried methanol and recrystalized from dried methanol. Satisfactory elemental and NMR analyses were obtained for $LacC_n$ and we successfully produced new type micellar surfactants for the first time.[17]

The hybrid liposomes were prepared by dissolving DMPC, Tween 20 and $LacC_n$ (DMPC : Tween 20 : $LacC_n$ = 65 : 7 : 28) in phosphate-buffered saline with sonication (BRANSONIC Model B2210) at 90 W and 45 °C.

Secondly, we examined the morphology of the three-component hybrid liposomes composed of DMPC, Tween 20 and $LacC_n$ (DMPC : Tween 20 : $LacC_n$ = 65 : 7 : 28) on the basis of dynamic light scattering measurement and electron microscopy.[18] The results are shown in Fig. 2. Distribution of hydrodynamic diameter (d_{hy}) (A) and an electron micrograph (B) of the three-component hybrid liposomes show the presence of uniform and spherical vesicles with a diameter of 100 nm.

Furthermore, the inhibitory effects of the three-component hybrid liposomes composed of DMPC, Tween 20 and $LacC_n$ (n=12, 14, 16) (DMPC : Tween 20 : $LacC_n$ = 65 : 7 : 28) on the growth of two types of tumor cells (human glioma (U251) and human lung adenocarcinoma (RERF-LC-OK)) were examined. The cells were cultured in a 5% CO_2 incubator at 37 °C. The inhibitory effect of hybrid liposomes on the growth of the cells was evaluated by calculating $A_{Mean}/A_{Control}$, where A_{Mean} and $A_{Control}$ denote the absorbance of water-soluble formazan in the presence and absence of the hybrid liposomes, respectively on the basis of the WST-1 method.[19] The

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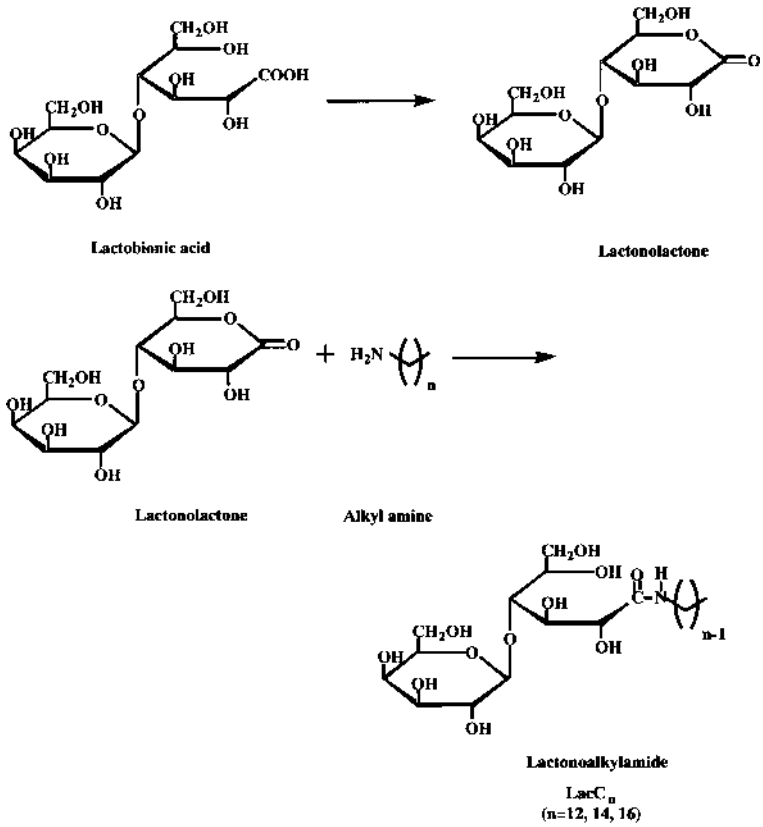

Fig. 1. Scheme for the Synthesis of $LacC_n$

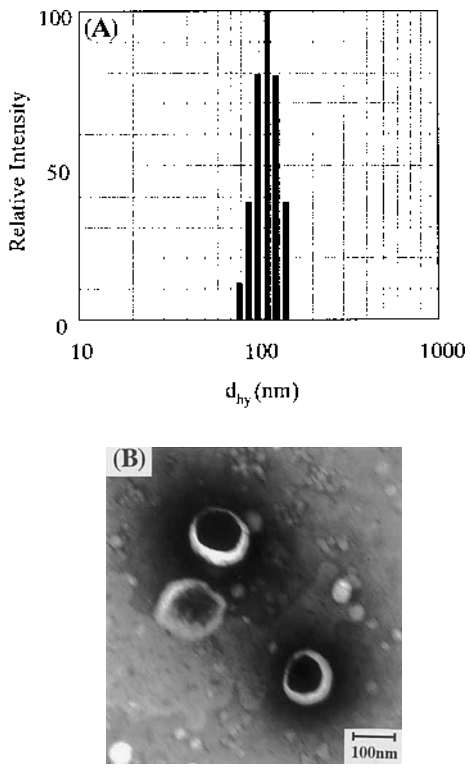

Fig. 2. (A) Distribution of Hydrodynamic Diameter (d_{hy}) and (B) an Electron Micrograph of the Three-Component Hybrid Liposomes Composed of DMPC, Tween 20, and $LacC_{14}$

results are shown in Fig. 3.

No significant inhibitory effects of any individual component (DMPC, Tween 20, Lac_{12}) or the two-component hybrid liposomes (HL) composed of DMPC and Tween 20 on the growth of tumor cells examined were obtained. It is noteworthy that highly inhibitory effects of the three-component hybrid liposomes composed of DMPC, Tween 20 and $LacC_n$ (n=12, 14, 16) (DMPC : Tween 20 : $LacC_n$=65 : 7 : 28) on the growth of tumor cells employed in this study were observed. These results suggest that the three-component hybrid liposomes composed of DMPC, Tween 20 and $LacC_n$ might be effective for specific recognition between a sugar group of $LacC_n$ and tumor cell membrane. It is attractive that the remarkably high inhibitory effect of the three-component hybrid liposomes composed of DMPC, Tween 20 and $LacC_{16}$ was attained on the growth of lung adenocarcinoma (RERF-LC-OK) cells, although the clear stock solution of $LacC_{16}$ micellar surfactants was not obtained.

The safety of the three-component hybrid liposomes (DMPC : Tween 20 : $LacC_n$=65 : 7 : 28) having the inhibitory effect was examined using normal mice. Four mice were assigned to each group by the stratified continuous randomization method and treated intravenously with hybrid liposomes (DMPC : Tween 20 : $LacC_n$=65 : 7 : 28) in single doses of 41—203 mg/kg (weight of DMPC). The mice were weighed during the experimental period. The measured weights of the mice after administering the hybrid liposomes increased in the same manner as the control group. All the mice were anesthetized after one week and anatomized. Kidney, spleen, liver, heart and lung of the mice were measured and relative organ weights of the mice were the same as the control

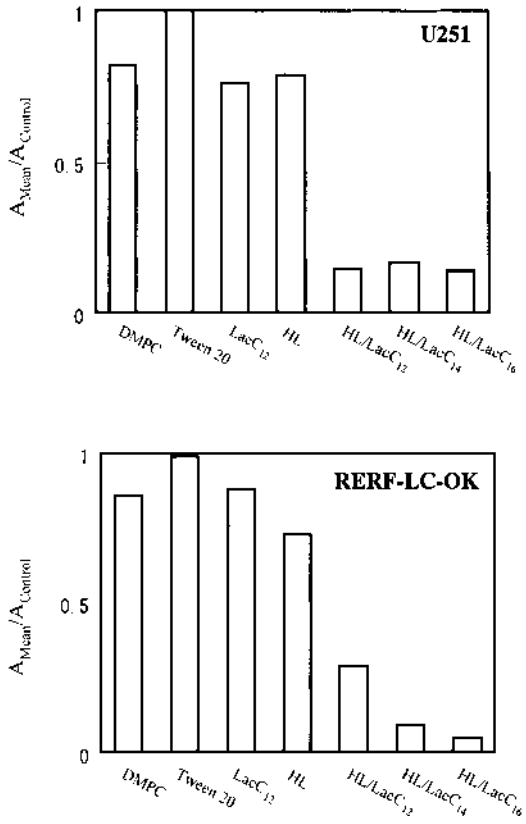

Fig. 3. Inhibitory Effects of Hybrid Liposomes Composed of DMPC, Tween 20, and $LacC_n$ (n=12, 14, 16) (DMPC : Tween 20 : $LacC_n$=65 : 7 : 28) on the Growth of U251 and RERF-LCOK Cells after Incubation for 48 h *in Vitro*

HL; hybrid liposomes composed of DMPC and Tween 20, (DMPC : Tween 20= 90 : 10). Initial cell number: 1.0×10^4 cells/ml, [DMPC]=3.0×10^{-4} M, [Tween 20]= 3.3×10^{-5} M, [$LacC_n$]=1.3×10^{-4} M. Concentration indicated are those in medium.

group.

In conclusion, high inhibitory effects of the three-component hybrid liposomes composed of DMPC, Tween 20, and $LacC_n$ (DMPC : Tween 20 : $LacC_n$=65 : 7 : 28) on the growth of glioma (U251) and lung adenocarcinoma (RERF-LC-OK) cells were obtained *in vitro* without any drug. Especially, it is highly significant that the remarkably high inhibitory effect was obtained for lung adenocarcinoma cells with the three-component hybrid liposomes composed of DMPC, Tween 20 and $LacC_{16}$. The recognition between sugar group of $LacC_n$ and tumor cell membrane would be important for inhibitory effects on the growth of tumor cells. We are now investigating the recognizant effects at the molecular level and the death process of tumor cells.

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References and Notes

1) Lasic D., "Liposomes: from Physics to Application," Elsevier, the Netherlands, 1993, pp. 261—286.
2) Ueoka R., Matsumoto Y., Moss R. A., Swarup S., Sugii A., Harada K., Kikuchi J., Murakami Y., *J. Am. Chem. Soc.*, **110**, 1588—1595 (1988).
3) Ueoka R., Matsumoto Y., Oyama H., Takekuma H., Ito M., Iwahara M., *Chem. Pharm. Bull.*, **36**, 4640—4643 (1988).
4) Ueoka R., Matsumoto Y., *Bio Industry*, **10**, 221—227 (1993).
5) Matsumoto Y., Imamura C., Ito T., Taniguchi C., Ueoka R., *Biol. Pharm. Bull.*, **18**, 1456—1458 (1995).
6) Matsumoto Y., Kato T., Kemura Y., Tsuchiya M., Yamamoto M., Ueoka R., *Chem. Lett.*, **1999**, 53—54.
7) Matsumoto Y., Kato T., Iseki I., Suzuki H., Nakano K., Iwahara M., Ueoka R., *Bioorg. Med. Chem. Lett.*, **9**,1937—1940 (1999).
8) Kanno A., Kodama R., Terada Y., Matsumoto Y., Ueoka R., *Drug Delivery System*, **13**, 101—105 (1998).
9) Kanno A., Terada Y., Tsuzaki K., Matsumoto Y., Ueoka R., *Drug Delivery System*, **14**, 37—42 (1999).
10) Ueoka R., Matsumoto Y., Kanno A., Tsuzaki K., Ichihara H., *Biol. Pharm. Bull.*, **23**, 1262—1263 (2000).
11) Kitamura I., Kochi M., Matsumoto Y., Ueoka R., Kuratsu J., Ushio Y., *Cancer Res.*, **56**, 3986—3992 (1996).
12) Gabius H. J., Engelhardt R., Hellmann T., Midoux P., Monsigny M., Nagel G. A.,Vehmeyer K., *Anticancer Res.*, **7**, 109—118 (1987).
13) Matsumoto Y., Kato T., Suzuki H., Hirose S., Naiki Y., Hirashima M., Ueoka R., *Bioorg. Med. Chem. Lett.*, **10**, 2617—2619 (2000).
14) Yamazaki N., Jigami Y., Gabius H. J., Kojima S., *Trends Glycosci. Glycotechnol.*, **13**, 319—329 (2001).
15) Varki A., *Glycobiology*, **3**, 97—130 (1993).
16) Kobayashi K., *Yukagaku*, **43**, 960—967 (1994).
17) Satisfactory elemental and NMR analyses were obtained for $LacC_n$. $LacC_{12}$: *Anal.* Calcd for $C_{24}H_{47}N$: C, 54.50; H, 8.99; N, 2.68. Found: C, 54.86; H, 8.95; N, 2.67. ^{1}H-NMR (270 MHz, DMSO-d_6) δ: 0.86 (3H, t, J=6.4 Hz, CH_3), 1.24 (18H, br s, CH×9), 1.39 (2H, m, CH_2), 3.06 (2H, m, CH_2), 3.23—3.79 (10H, m, CH×6, CH_2×2), 4.04—4.12 (3H, m, CH×2, OH), 4.26 (1H, d, J=6.6 Hz, CH), 4.49 (2H, s, OH×2), 4.68 (1H, t, J=5.4 Hz, OH), 4.79 (1H, br s, OH), 4.80 (1H, br s, OH), 5.17 (2H, s, OH×2), 7.57 (1H, t, J=5.9 Hz, NH). $LacC_{14}$: *Anal.* Calcd for $C_{26}H_{51}N$: C, 56.40; H, 9.28; N, 2.53. Found: C, 56.26; H, 9.26; N, 2.53. ^{1}H-NMR (270 MHz, DMSO-d_6) δ: 0.85 (3H, t, J=6.6 Hz, CH_3), 1.24 (22H, br s, CH_2×11), 1.40 (2H, m, CH_2), 3.06 (2H, m, CH_2), 3.22—3.77 (10H, m, CH×6, CH_2×2), 3.94—4.13 (3H, m, CH×2, OH), 4.26 (1H, d, J=6.9 Hz, CH), 4.43—4.53 (2H, m, OH×2), 4.67 (1H, t, J=5.6 Hz, OH), 4.79 (2H, d, J=5.3 Hz, OH×2), 5.11—5.20 (2H, m, OH×2), 7.57 (1H, t, J=5.9 Hz, NH). $LacC_{16}$: *Anal.* Calcd for $C_{28}H_{55}N$: C, 57.91; H, 9.55; N, 2.48. Found: C, 57.83; H, 9.47; N, 2.41. ^{1}H-NMR (270 MHz, DMSO-d_6) δ: 0.85 (3H, t, J=6.6 Hz, CH_3), 1.23 (26H, br s, CH_2×13), 1.39 (2H, m, CH_2), 3.06 (2H, m, CH_2), 3.22—3.76 (10H, m, CH×6, CH_2×2), 3.94—4.12 (3H, m, CH×2, OH), 4.26 (1H, d, J=6.9 Hz, CH), 4.43—4.52 (2H, m, OH×2), 4.67 (1H, t, J=5.6 Hz, OH), 4.78 (2H, d, J=5.0 Hz, OH×2), 5.15 (2H, d, J=4.3 Hz, OH×2), 7.57 (1H, t, J=5.9 Hz, NH).
18) The dynamic light scattering measurements were performed with a Brookhaven BI 90 particle sizer as described in ref 2. The electron micrographs were obtained by mean of negative staining method with an electron microscope (Hitachi Model 300).
19) Ishiyama M., Shiga M., Sasamoto K., Mizoguchi M., He P. G., *Chem. Pharm. Bull.*, **41**, 118—120 (1993).