Inhibitory Effects of Hybrid Liposomes Containing Lactose Surfactants on the Growth of Tumor Cells

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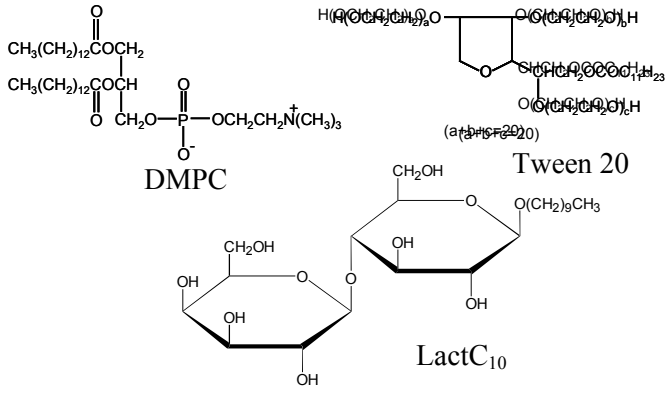
Abstract: - Three-component hybrid liposomes (THL) composed of L-α-dimyristoylphosphatidylcholine, polyoxyethylene sorbitane monolaurate (Tween 20), decyl-β-lactoside ($LactC_{10}$) were found to be highly effective for inhibiting the growth of human hepatoma (Hep-G2) cells without any drug.

Key-Words: - Liposome, Lactose Surfactant, Fixed Aqueous Layer, Apoptosis, Antitumor

1 Introduction

It is well known that saccharides play important roles in adhering to cells, transmitting information, recognizing molecules on the cell membranes through receptors including lectin [1]. Hydration of saccharides with hydrogen bonds provides stability to the structure of water. Recently, the preparation and characterization of glyco-liposomes have been reported [2, 3]. On the other hand, new-type hybrid liposomes (HL) composed of vesicular and micellar molecules have been produced [4]. HL can be prepared by sonication of vesicular and micellar molecules in a buffer solution. The physical properties of HL such as shape, size, membrane fluidity, and the temperature of phase transition can be controlled by changing the constituents and compositional ratios [5]. HL without drugs inhibited the proliferations of various tumor cells along with apoptosis in vitro and in vivo [6-9]. In addition, highly specific inhibitory effects of three-component hybrid liposomes including sucrose surfactants on the growth of glioma cells were obtained [3]. However, there is no report on glyco-liposomes using lactose surfactants.



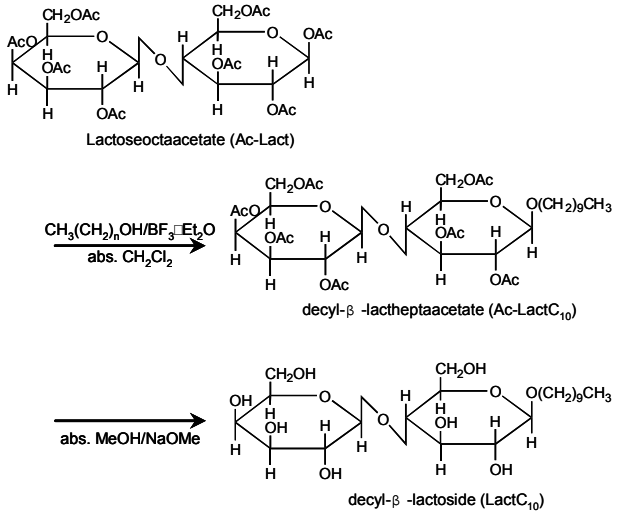
Lactose is a disaccharide composed of D-galactose and plays an important role in molecular recognition in vivo [10]. In this study, we report on inhibitory effects of three-component hybrid liposomes composed of dimyristoylphosphatidylcholine (DMPC), polyoxyethylene sorbitane monolaurate (Tween 20), and decyl-β-lactoside ($LactC_{10}$) on the growth of tumor cells in vitro.

2 Results and Discussion

2.1 Synthesis of lactose surfactant

We prepared decyl-β-lactoside ($LactC_{10}$) from decyl-β-lactoheptaacetate ($Ac\text{-}LactC_{10}$) [11, 12], which was obtained by glycosylation and deacetylation of lactoseoctaacetate (Ac-Lact) (Scheme 1). Ac-Lact (4.5 mmol) and 1-decanol (6.8 mmol) were dissolved in dry dichloromethane (40 ml) under a nitrogen atmosphere. After the addition of boron trifluoride diethyl etherate (5.9 mmol), the solution was stirred at room temperature for 2 h. The reaction mixture was poured into saturated solution (40 ml) of sodium hydrogen carbonate, the organic layer was separated and aqueous layer was extracted twice with dichloromethane (40 ml). The combined organic phases were washed twice with water (40 ml) and dried on magnesium sulphate over night. After removal of the solvent, crude syrup-like product was obtained. Column chromatography on silica gel 60 (2.5×45 cm) with n-hexane/ethyl acetate (1 : 1) yielded pure decyl-β-lactoheptaacetate ($Ac\text{-}LactC_{10}$). We successfully produced new type lactose surfactants. Satisfactory analytical date were obtained

for $LactC_{10}$. mp 185.6-186.5˚C Anal. Calcd for $C_{22}H_{42}O_{11}$: C, 55.88; H, 9.13. Found: C, 55.45, H, 9.13. ^{1}H-NMR (270 MHz, DMSO-d_6)δ0.85 (3H, t, J = 6.92 Hz, CH_3), 1.25 (14H, br s, CH_2×7), 1.51 (2H, m, CH_2), 2.98 (1H, m, CH), 3.27-3.76 (13H, m, CH×9, CH_2×2), 4.15 (1H, d, J = 7.92 Hz, CH), 4.18 (1H, d, J = 7.91 Hz, CH), 4.54 (2H, m, OH×2), 4.66 (2H, s, OH×2), 4.80 (H, br s, OH), 5.08 (H, s, OH), 5.10 (H, s, OH).



Scheme 1 Synthesis of decyl-β-lactoside

2.2 Inhibitory effects of three-component hybrid liposomes

Three-component hybrid liposomes (DMPC : Tween 20 : $LactC_{10}$ = 65 : 7 : 28) were prepared by sonication (Velvo VS-N300, 300W) of a mixture containing phospholipids (DMPC), micellar surfactants (Tween 20), and lactose surfactants ($LactC_{10}$) in phosphate-buffered saline at 45 ˚C in a nitrogen atmosphere and, followed by filtration with a 0.45 μm filter. We examined the effect of the three-component hybrid liposomes (DMPC : Tween 20 : $LactC_{10}$ = 65 : 7 : 28) (THL) on the growth of human hepatoma (Hep-G2) cells on the basis of the WST-1 assay [13]. The tumor cells (5.0×10^5 viable cells/ml) were cultured for 48 h in a humidified 5% CO_2 incubator at 37 ˚C after adding the sample solutions. Then WST-1 solutions were added to the cells and the absorbance at a wavelength of 450 nm was measured by spectrophotometer. The inhibitory concentration was evaluated by $A_{Mean}/A_{Control}$, where A_{Mean} and $M_{Control}$ denote the absorbance of water-soluble formazan, which was useful as an indicator of cell viability, in the presence and absence of sample solutions, respectively. The results are shown in Fig. 1. It is noteworthy that highly inhibitory effects of THL on the growth of Hep-G2 cells were obtained, On the other hands, no significant inhibitory effects by any individual component (DMPC, Tween 20, $LactC_{10}$) or the two-component hybrid liposomes (HL) composed of DMPC and Tween 20 on the growth of Hep-G2 cells were obtained. No inhibitory effects of THL on the growth human hepatic cells were obtained.

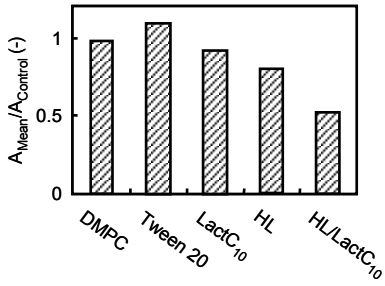


Fig. 1 Inhibitory effects of three-component hybrid liposomes composed of DMPC, Tween 20 and $LactC_{10}$ on the growth of Hep-G2 cells after incubation for 48 h in vitro. HL; hybrid liposomes composed of DMPC and Tween 20. [DMPC]=300μM, [Tween 20]=33μM, [$LactC_{10}$]=129μM (DMPC : Tween 20 : $LactC_{10}$ = 65 : 7 : 28)

2.3 Thickness of fixed aqueous layer of three-component hybrid liposomes

We examined the thickness of fixed aqueous layer of the three-component hybrid liposomes from zeta potential. Zeta potential were measured on the basis of laser doppler flowmetry method [14] using an electrophoretic light scatteing apparatus (Otsuka ELS-8000). It is noteworthy that the thickness of the fixed aqueous layer of THL (0.58 nm) was about twice that of HL composed of DMPC and Tween 20 (0.31 nm) (Fig. 2). This result suggests that the inhibitory effects of THL should be related to hydration of lactose surfactants incorporated into liposomes.

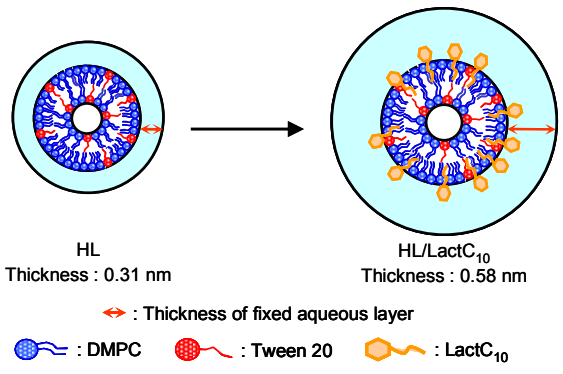


Fig. 2 Schematic representation of fixed aqueous layer of three-component hybrid liposomes.

2.4 Induction of apoptosis by three-component hybrid liposomes

We examined induction of apoptosis by THL for Hep-G2 cells using double staining method with quinolinium-4-[(3-methyl-2(3H)-benzoxazolylidene) methyl]-1-[3-(trimethylammonio)propyl]-diiodide (YOPRO-1) [Ex./Em. (nm) = 491/509] and Propidium Iodide (PI) [Ex./Em. (nm) = 493/635] [15]. Fluorescence micrographs of Hep-G2 cells after the treatment with THL are shown in Fig. 3. Apoptotic and necrotic cells are dyed in green (YOPRO-1) and red or orange color (PI), respectively. Interestingly, cells were dyed in green after adding THL, indicating that those liposomes could induce apoptosis for Hep-G2 cells.

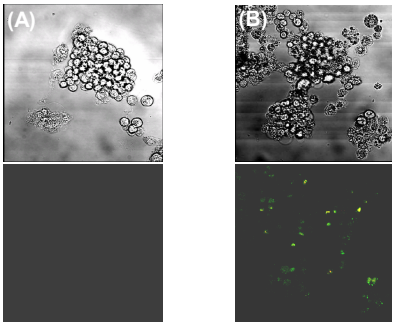


Fig. 3 Fluorescence micrographs of Hep-G2 cells treated with two-component hybrid liposomes (A) and three-component hybrid liposomes (B) for 24 h. HL; hybrid liposomes composed of DMPC and Tween 20. [DMPC]=300μM,[Tween20]=33μM,[LactC_{10}]=129μM (DMPC : Tween 20 : LactC_{10} = 65 : 7 : 28)

3 Conclusion

High inhibitory effects of THL composed of DMPC, Tween 20, and LactC_{10} (DMPC : Tween 20 : LactC_{10} = 65 : 7 : 28) on the growth of human hepatoma (Hep-G2) cells were obtained in vitro without any drug. Furthermore, these liposomes induced apoptosis for Hep-G2 cells. It is noteworthy that the thickness of fixed aqueous layer of THL was about twice that of two-component hybrid liposomes. These results suggest that THL including lactose surfactants should be effective for inhibiting the growth of Hep-G2 cells through apoptosis in relation to hydration of saccharides.

Acknowledgment

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