Intracellular Responses of Hybrid Liposomes against Leukemia Cells Related to Apoptosis with Antitumor Activity

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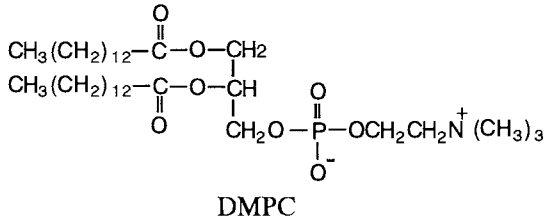
Intracellular responses of hybrid liposomes against leukemia cells related to apotosis were observed for the first time along with the highly inhibitory effects.

With the advancement of medicinal chemistry, gene engineering, and molecular biology in recent years, various cell functions, such as intracellular migration of substances and transmission of signals, have been increasingly discussed on a molecular level. In the field of cancer research, the interrelation between cancer and apoptosis (programmed cell death), and the development of novel anticancer drugs, have been subjects of intensive study.

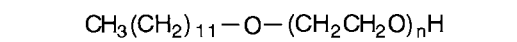
Hybrid liposomes, first developed by Ueoka *et al.*, can be prepared by simply ultrasonicating a mixture of vesicular and micellar molecules in the buffer solution; shape, size, and the temperature of phase transition of these liposomes can be controlled by changing the constituents and compositional ratios.[1] Usefulness of hybrid liposomes has been widely expected as a new material in the medical field because of their apparent bioadaptability, easiness of preparation than the ordinary methods, and no contamination with any organic solvents. It has been reported that hybrid liposomes demonstrated remarkable inhibitory effects on lymphoma cells,[2-5] and acute toxicity tests using normal rats indicated that hybrid liposomes were free from any toxic action.[6] Furthermore, the hybrid liposomes including antitumor drugs have been found to have a highly inhibitory effect on the growth of glioma in vitro and in vivo.[7] The significantly prolonged survival was obtained using mice model of carcimona in vivo.[8]

In this study, we report for the first time on intracellular responses of the hybrid liposomes having inhibitory action toward the growth of tumor cells related to apoptosis.

Firstly, we examined the induction of apoptosis for human promyelocytic leukemia (HL-60) cells by the hybrid liposomes composed of dimyristoylphosphatidylcholine (DMPC) and polyoxyethylene dodecyl ether ($C_{12}(EO)_n$; n = 4, 10, 23). The results are shown in Figure 1. It is noteworthy that exposure of HL-60 cells to the hybrid liposomes of DMPC/$C_{12}(EO)_{10}$ caused DNA fragmentation characteristic of apoptosis. Furthermore, the



DMPC



$C_{12}(EO)_n$ (n = 4, 10, 23)

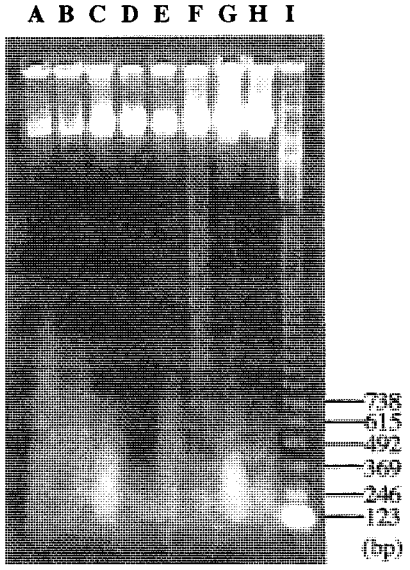


Figure 1. Agarose gel electrophoresis of DNA from HL-60 cells treated with hybrid liposomes (A : Control, B : DMPC, C : $C_{12}(EO)_4$, D : DMPC/10 mol% $C_{12}(EO)_4$, E : $C_{12}(EO)_{10}$, F : DMPC/10 mol% $C_{12}(EO)_{10}$, G : $C_{12}(EO)_{23}$, H : DMPC/10 mol% $C_{12}(EO)_{23}$, I : DNA marker). Initial cell number : 1.0×10^4 cells/ml, [DMPC] = 7.5×10^{-5} M (1 M = 1 mol dm^{-3}), [$C_{12}(EO)_n$] = 8.3×10^{-6} M, culture time : 24 h.

induction of apoptosis by the hybrid liposomes in HL-60 cells was verified on the basis of fluoresence microscopy with the fragmented DNA-staining method (data not shown). It is also attractive that the almost completely inhibitory effect (96 %)[9] was attained by employing the hybrid liposomes of

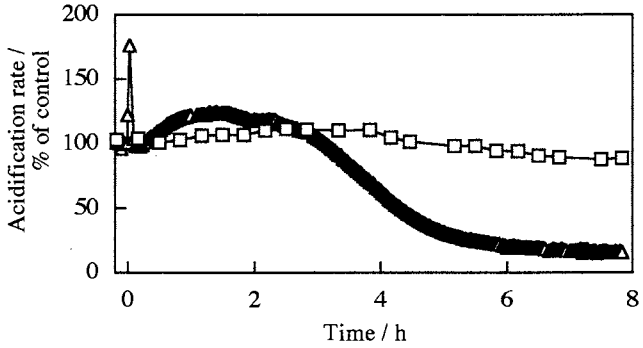


Figure 2. Acidification rate for HL-60 cells treated with hybrid liposomes composed of 90 mol% DMPC and 10 mol% $C_{12}(EO)_{10}$ (□: control). [DMPC] = 5.0×10^{-4} M, [$C_{12}(EO)_{10}$] = 5.6×10^{-5} M.

DMPC/$C_{12}(EO)_{10}$. On the other hand, the inhibitory effects were not so remarkable for other hybrid liposomes (DMPC/$C_{12}(EO)_4$ and DMPC/$C_{12}(EO)_{23}$), DMPC liposomes, and $C_{12}(EO)_n$ micelles.

Secondly, we examined intracellular responses of the hybrid liposomes composed of DMPC and $C_{12}(EO)_{10}$ against HL-60 cells related to apoptosis. Intracellular responses of HL-60 cells after the addition of hybrid liposomes were measured by the Cytosensor microphysiometer (Molecular Devices).[10] The results are shown in Figure 2. A rapid and transient increase in the acidification rate was observed immediately after adding hybrid liposomes to the culture medium. This transient increase in acidification is probably due to a massive release of protons from inside to outside of cells following the opening of ion channels. After the initial response, the acidification rate gradually increased, to reach a maximum value, and thereafter it slowly decreased. It is presumed that the increase in acidification was caused by activation of some membrane proteins in the HL-60 cells after their fusion with hybrid liposomes, and the decrease in acidification was caused by subsequent apoptosis of HL-60 cells.[11] It was suggested that the almost constant value of acidification rate for 6 h might reach to the completion of apoptosis. Relative DNA contents and the time course of accumulation of DNA fragmented by DMPC/10 mol% $C_{12}(EO)_{10}$ hybrid liposomes in the same concentration as in Figure 2 are shown in Figure 3.[12] The DNA of HL-60 cells was found to be almost completely fragmented 6 h after the addition of hybrid liposomes, indicating the completion of apoptosis. It is noteworthy that the close agreement of completion time for the apoptosis between microphysiometry and flowcytometry experiments was observed.

In conclusion, it is attractive that intracellular responses of hybrid liposomes against leukemia cells (HL-60) related to apotosis were observed for the first time along with the highly inhibitory effects on the growth of HL-60 cells.

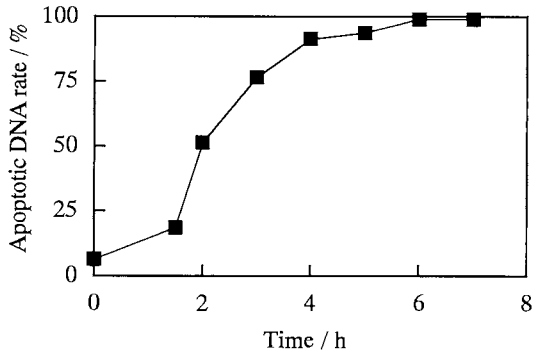


Figure 3. Apoptotic DNA rate for HL-60 cells treated with hybrid liposomes composed of 90 mol% DMPC and 10 mol% $C_{12}(EO)_{10}$. [DMPC] = 5.0×10^{-4} M, [$C_{12}(EO)_{10}$] = 5.6×10^{-5} M.

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9 The cells (initial cell number : 1.0×10^4 cells/ml) were cultured for 4 d in a 5 % CO_2 incubator at 37 °C after adding the hybrid liposomes ([DMPC] = 7.5×10^{-5} M, [$C_{12}(EO)_n$] = 8.3×10^{-6} M). The inhibitory effects of hybrid liposomes on the growth of tumor cells were evaluated by $100(N_a-N_p)/N_a$, where N_a and N_p denote the live cell numbers in the absence and presence of the hybrid liposomes, respectively. The hybrid liposomes were prepared by dissolving both DMPC and $C_{12}(EO)_n$ in phosphate-buffered saline with sonication (BRANSONIC Model B2210 apparatus, 90 W) at 45 °C for 5 min.
10 The Cytosenser microphysiometer measures the change in extracellular acidification rate resulting either from alterations in the energy demand made on the cells as they respond to the effector agents or from alterations in sodium-hydrogen exchange across the cell membrane described in J.W. Parce, J.C. Owicki, K.M. Kercso, G.B. Signal, H.G.Wada, V.C. Muir, L.J. Bousse, K.L. Ross, B.I. Sikic, and H.M. McConnell, *Science*, **246**, 243 (1989).
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12 The DNA content was determined in a flow cytometer (Epics XL system II, Coulter) with the propidium iodide staining method.