REMARKABLE INHIBITORY EFFECTS OF HYBRID LIPOSOMES ON THE GROWTH OF HL-60 CELLS COUPLED TO INDUCTION OF APOPTOSIS

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The hybrid liposomes (90 mol% DMPC/10 mol% C_{12}(EO)_{10} or C_{12}(EO)_{12}) have a highly inhibitory effect on the growth of tumor cells (HL-60). The induction of apoptosis by the hybrid liposomes in HL-60 cells was revealed on the basis of flow cytometry and DNA electrophoresis.

KEY WORDS liposome; apoptosis; in vitro antitumor activity

Liposomes have recently attracted attention in connection with reducing the toxicity of antitumor drugs.\textsuperscript{1} We have recently produced new-type hybrid liposomes composed of vesicular and micellar molecules. The physical properties of these liposomes, such as size, membrane fluidity, phase transition temperature, and hydrophobicity can be controlled by changing the composition of hybrid liposomes.\textsuperscript{2} In the course of our study, remarkably high inhibitory effects of hybrid liposomes without drugs on lymphoma growth in vitro have been obtained.\textsuperscript{3-6} 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.\textsuperscript{7} No toxicity of the hybrid liposomes was observed in normal rats in vivo without any side effects.\textsuperscript{8}

On the other hand, it has been reported that many kinds of antitumor drugs induce the apoptosis of various tumor cells.\textsuperscript{9, 10} However, there has been no report of the induction of apoptosis by liposomes without drugs.

In this study, we examined the inhibitory effect of hybrid liposomes composed of dimyristoyl-phosphatidylcholine (DMPC) and polyoxyethylene dodecyl ether (C_{12}(EO)\textsubscript{n}; \textit{n} = 4, 8, 10, 12, 23) on the growth of human promyelocytic leukemia (HL-60) cells in vitro. The cells were cultured for 4 days in a 5% CO\textsubscript{2} incubator at 37°C after adding the hybrid liposomes. The inhibitory effects of hybrid liposomes on the growth of tumor cells were evaluated by 100(N\textsubscript{a} - N\textsubscript{p})/N\textsubscript{a}, where N\textsubscript{a} and N\textsubscript{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)\textsubscript{n} in phosphate-buffered saline with sonication (BRANSONIC Model B2210 apparatus, 90W) at 45°C for 5 min.

\[
\begin{array}{c}
\text{CH}_3(\text{CH}_2)_{12}-\text{C}^\text{−O}^\text{−CH}_2 \\
\text{CH}_3(\text{CH}_2)_{12}-\text{C}^\text{−O}^\text{−CH} \\
\text{CH}_2^\text{−O}^\text{−P}^\text{−OCH}_2\text{CH}_2\text{N(CH}_3)\text{\textsubscript{3}} \\
\text{DMPC} \\
\end{array}
\begin{array}{c}
\text{O} \\
\text{O} \\
\text{O} \\
\text{O} \\
\end{array}
\begin{array}{c}
\text{CH}_3(\text{CH}_2)_{11} \text{− O}^\text{− (CH}_2\text{CH}_2\text{O)}\text{\textsubscript{n}} \text{− H} \\
\text{C}_{12}(\text{EO})\text{\textsubscript{n}} \\
\end{array}
\]

First, the inhibitory effect of the hybrid liposomes composed of DMPC and C_{12}(EO)\textsubscript{n} on the growth of HL-60 cells was examined. The results are summarized in Table 1. The noteworthy

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aspects are as follows: (a) The inhibitory effects of the hybrid liposomes composed of DMPC and C_{12}(EO)_{4} are moderately enhanced as compared with those of the liposomes of DMPC. (b) The inhibitory effects of DMPC/C_{12}(EO)_{8} and DMPC/C_{12}(EO)_{23} hybrid liposomes were fairly enhanced. (c) Almost completely inhibitory effects were attained by employing the hybrid liposomes of DMPC/C_{12}(EO)_{10} and DMPC/C_{12}(EO)_{12}. These results suggest that the hydrophilic-hydrophobic balance in polyoxyethylene alkyl ethers is important for the enhancement of the inhibitory effect on the growth of tumor cells, as previously reported.\textsuperscript{6}

Table 1. Inhibitory Effects of Hybrid Liposomes Composed of 90 mol% DMPC and 10 mol% C_{12}(EO)_{n} on the Growth of HL-60 Cells

<table>
<thead>
<tr>
<th>Sample</th>
<th>Inhibitory Effect (%)</th>
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<tbody>
<tr>
<td>DMPC</td>
<td>25</td>
</tr>
<tr>
<td>DMPC/10mol%C_{12}(EO)_{4}</td>
<td>64 (0)</td>
</tr>
<tr>
<td>DMPC/10mol%C_{12}(EO)_{8}</td>
<td>89 (77)</td>
</tr>
<tr>
<td>DMPC/10mol%C_{12}(EO)_{10}</td>
<td>96 (83)</td>
</tr>
<tr>
<td>DMPC/10mol%C_{12}(EO)_{12}</td>
<td>99 (75)</td>
</tr>
<tr>
<td>DMPC/10mol%C_{12}(EO)_{23}</td>
<td>79 (3)</td>
</tr>
</tbody>
</table>

\[ [\text{DMPC}] = 7.5 \times 10^{-5} \text{M}, \ [\text{C}_{12}(\text{EO})_{n}] = 8.3 \times 10^{-6} \text{M} \]

Initial cell number: 1.0 \times 10^{4} \text{ cells/ml}.

Values in the parentheses are those of C_{12}(EO)_{n} micelles ([C_{12}(EO)_{n}] = 8.3 \times 10^{-6} \text{M}).

The inhibitory effects have maximum errors of \pm 6\%.

Second, the morphological changes in HL-60 cells were examined using a flow cytometer.\textsuperscript{11} The shrinkage of cells was observed by adding the hybrid liposomes, as shown in Fig. 1. On the basis of DNA agarose gel electrophoresis, it was also found that exposure of HL-60 cells to hybrid liposomes caused DNA fragmentation characteristic of apoptosis (Fig. 2). These results suggest that

![Cytograms of HL-60 Cells after Treatment with the Hybrid Liposomes Composed of 90 mol% DMPC and 10 mol% C_{12}(EO)_{12} for 3 h](image-url)
DMPC/10 mol% C_{12}(EO)_{10} or C_{12}(EO)_{12} hybrid liposomes induce the apoptosis of HL-60 cells.

In conclusion, it is noteworthy that the growth inhibition of human promyelocytic leukemia cells was attained by employing hybrid liposomes composed of 90 mol% DMPC and 10 mol% C_{12}(EO)_{10} or C_{12}(EO)_{12} in vitro and that the hybrid liposome-induced apoptotic cell death of HL-60 cells was seen for the first time. By using at least two criteria for the assessment of the cell death mechanism, cell morphology and the pattern of DNA degradation, we have shown that hybrid liposomes lead to apoptosis in human leukemic cells.\(^{12}\)

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**REFERENCES AND NOTES**

11) The flow cytometric measurements were performed with a BIO RAD BRYTE-HS flow cyrometer and Xe-Hg lamp as a light source. The scattering light of non-staining cells was measured.

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