Nanoparticles of Hybrid Liposomes for the Inhibition of Breast Tumor Growth along with Apoptosis

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Hybrid liposomes (HLs) composed of L-α-dimyristoylphosphatidylcholine and polyoxyethylene (25) dodecyl ether having 80 nm in diameter were produced. It is noteworthy that the therapeutic effects of HLs along with apoptosis without side effect were obtained using xenograft mice models of human breast cancer in vivo for the first time.

Liposomes are closed vesicles that are formed when phospholipids (constituents of biological membranes) are dispersed in water at relatively low concentrations.[1] These liposomes have been studied for chemical and medical applications. For example, liposomes have contributed significantly to drug delivery and for analyzing the cellular function, owing to their mimicry of biological membranes and closed properties.[2,3] We have produced hybrid liposomes (HLs) composed of vesicular and micellar molecules.[4] The physical properties of HLs such as shape, size, membrane fluidity, and the temperature of phase transition can be controlled by changing the constituents and compositional ratios. We have applied HLs to enzymological and medical applications. In relation to membrane-bound enzymes models, we have succeeded in the perfect steric control for the enatioselective hydrolysis of amino acid esters with HLs.[5]

On the other hand, chemotherapy with anticancer drugs for the whole body is one of the most widely used treatments for breast cancer. However, most anticancer drugs are accompanied by severe side effects.[6] So, drugs without side effects are necessary to lead to high QOL (quality of life) for patients. Molecular targeted therapy for tumors has recently attracted attention in connection with reducing the toxicity of anticancer drugs. Inhibitory effects of HLs composed of L-α-dimyristoylphosphatidylcholine (DMPC) and polyoxyethylene (20) sorbitan monolaurate (Tween 20) including antitumor drugs such as 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) have been observed on the growth of glioma cells in vitro and in vivo.[7] HLs composed of DMPC and polyoxyethylene alkyl ethers without any drugs have remarkable inhibitory effects on the growth of tumor cells in vitro[8–10] and in vivo.[11,12] Successful clinical chemotherapy with drug-free HLs to patients with lymphoma has been reported after passing the committee of bioethics.[12] However, therapeutic effects in vivo of HLs for human breast tumor (MDA-MB-453) cells were not elucidated. In this study, we investigated therapeutic effects of HLs composed of DMPC and polyoxyethylene (25) dodecyl ether ($C_{12}(EO)_{25}$) without any drugs using xenograft mice model of human breast cancer in vivo.

Morphology of HLs was examined on the basis of dynamic light scattering measurements and electron microscopy.[13] HLs were prepared by sonication (VELVO VS-N300, 300W) of a mixture containing 95 mol % DMPC and 5 mol % $C_{12}(EO)_{25}$ in 5% glucose solution at 45 °C, followed by filtration with a 0.20-μm cellulose acetate filter. Hydrodynamic diameter (d_{hy}) of HLs was about 80 nm as shown in Figure 1, which remained stable for more than one month. It is worthy to note that HLs having 80 nm in diameter could avoid the reticular endothelial system.[14]

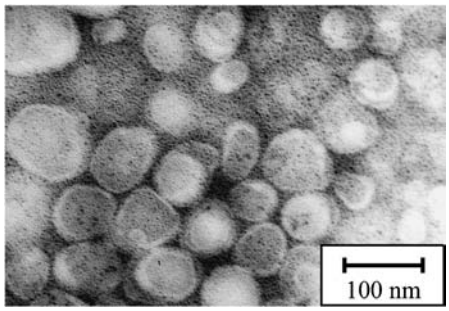

Figure 1. An electron micrograph of HLs.

We examined the therapeutic effects of HLs using xenograft mice models after the inoculation of MDA-MB-453 cells in vivo. MDA-MB-453 cells were obtained from Riken Cell Bank. MDA-MB-453 cells (5.0×10^6 cells) suspended into matrigel were subcutaneously inoculated dorsally in mice (BALB/cAJcl-*nu*/*nu*, CLEA). After the inoculation of MDA-MB-453 cells for one week, the increases in tumor volume of mice were obtained, and the mice were randomly grouped on the basis of the tumor volume using the stratified randomization method. The number of mice was three in each group. HLs were intravenously administered once each day for two weeks from day 7 after the inoculation of MDA-MB-453 cells. The tumor volume was measured using vernier calipers and calculated using the equation of $V = 0.5 \times a^2 \times b$, where a and b denote the smallest and longest superficial diameter, respectively.[15] The results are shown in Figure 2a. Photographs of tumors in xenograft mice model are shown in Figure 2b. The tumor volumes in the treatment group were almost constant, although those in the control group increased. After the administration of HLs for two weeks, the median of tumor volume was 233 ± 61 and $458 \pm 32\,mm^3$ in the group of treatment and control, respectively. It is noteworthy that the remarkable suppression of tumor volume (50%) in mice inoculated MDA-MB-453 cells was obtained after the treatment with HLs for the first time.[16]

Moreover, we examined induction of apoptosis by HLs for breast tumor in xenograft mice using the TUNEL method.[17,18] The results are shown in Figure 3. Brown color was observed in the tumor cells of xenograft mice after the treatment with HLs, although the apoptotic cells were not observed in the control group. These results indicate that HLs have remarkable suppression effects on the growth of breast tumor along with apoptosis in vivo.

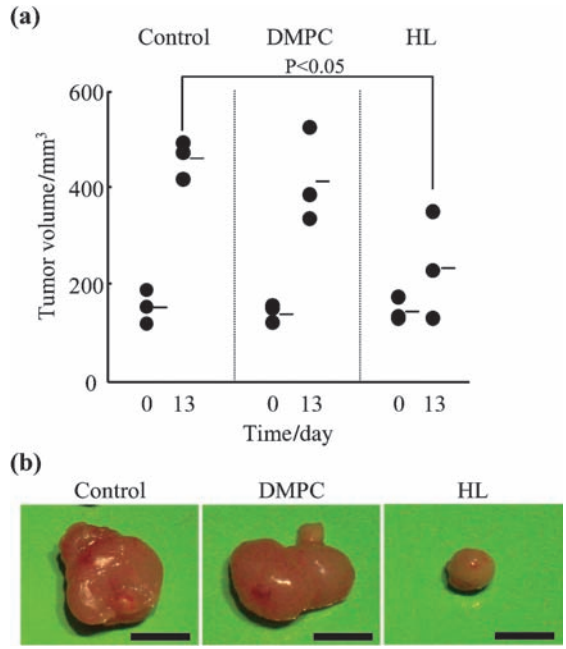

Figure 2. Suppression of tumor volume in xenograft model mice intravenously treated with HLs after subcutaneous inoculating MDA-MB-453 cells. (a) Tumor volume of xenograft mice model intravenously treated with HLs after subcutaneous inoculating MDA-MB-453. Dose for DMPC is 203 mg/kg. (b) Photographs of tumor in xenograft mice model intravenously treated with HLs after subcutaneous inoculating MDA-MB-453. The size of the bar is 5 mm.

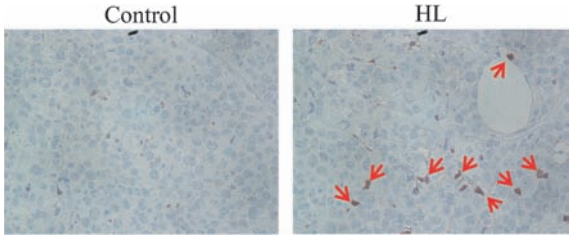

Figure 3. Micrographs of tissue sections in xenograft model mice using TUNEL method after the treatment with HLs. Dose for DMPC = 203 mg/kg.

Safety tests were carried out using normal mice (BALB/cAJcl, CLEA) after treatment intravenously with HLs for 14 days. No weight loss was observed in the mice. Hematological tests[19] and relative organ weights were examined. Red blood cells (RBC) were $454 \times 10^4/\mu L$ and $495 \times 10^4/\mu L$ for normal mice after the treatment with and without HLs, respectively, indicating that no abnormal findings were observed in the number of RBC for mice treated with HLs. The numbers of white blood cells (WBC) were $33 \times 10^2/\mu L$ and $29 \times 10^2/\mu L$ for treated and control groups, respectively. No abnormal findings were observed in the heart, lung, liver, and kidney. These results indicate that HLs should have no severe side effects in vivo.

In conclusion, we clearly demonstrated that therapeutic effects of HLs along with apoptosis without side effects were obtained for xenograft mice models of human breast tumor in vivo for the first time. The noteworthy aspects of this study are as follows: (a) Remarkable suppression of tumor volume in a xenograft mice model intravenously treated with HLs without drugs after the inoculation of MDA-MB-453 cells was verified in vivo. (b) Induction of apoptosis in tumors of xenograft mice treated with HLs was observed in micrographs on the basis of TUNEL method. The results of this study could be advantageous for the chemotherapy for patients with breast cancer in future clinical applications.

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