Therapeutic effects of hybrid liposomes for mouse models of adult T-cell leukemia/lymphoma in vivo

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ABSTRACT: Hybrid liposomes (HLs) composed 90 mol% L-α-dimyristoylphosphatidylcholine (DMPC) and 10 mol% polyoxymethylene(25) dodecyl ether (C₁₃EO₂₅), having a hydrodynamic diameter of 20-80 nm, were preserved for a period of one month. Remarkably high therapeutic effects were obtained in the mouse models of adult T-cell leukemia/lymphoma (ATL) after the treatment with HLs on the basis of relative organ weight; histological analysis of the liver tissue sections of the mouse models of ATL was done with hematoxylin-eosin (HE) and immunohistochemical staining. Furthermore, prolonged survival was obtained in the mouse models of ATL after the treatment with HLs. It is noteworthy that the therapeutic effect of HLs without any drugs in the mouse models of ATL was revealed on the basis of histological analysis and life prolongation rates for the first time in vivo. © Global Scientific Publishers 2012

KEYWORDS: Hybrid liposomes, Adult T-cell leukemia/lymphoma, mouse model, CD4, CD25, MT-4.

Adult T-cell leukemia/lymphoma (ATL) is a T-cell malignancy infected with human T-cell leukemia virus type 1 (HTLV-1) [1-3]. Clinical subtypes of ATL include acute, lymphoma, chronic and smoldering types. Common findings for patients with ATL include enlargement of peripheral lymph nodes, hepatomegaly and skin lesions. ATL has poor prognosis compared with B-cell lymphoma and peripheral T-cell one because of its resistance to anticancer agents. Combination chemotherapy [4, 5], stem cell transplantation [6] and molecular targeted agents [7-9] are widely used treatments for ATL. However, while chemotherapy agents kill tumor cells, they also damage normal ones. The survival of patients with ATL is still poor regardless of intensive chemotherapies. On the other hand, recent reports of retrospective analysis of stem cell transplantation for ATL showed improvement of the therapeutic outcome, although stem cell transplantation for ATL still had extremely poor prognosis [10]. Thus, alternative treatment strategies for ATL are needed.

Ueoka has produced hybrid liposomes (HLs) composed of vesicular and micellar molecules for the first time [11, 12]. 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. Inhibitory effects of HLs including antitumor drugs have been observed on the growth of glioma cells in vitro and in vivo [13]. HLs have been effective for inhibiting the growth of various tumor cells in vitro and in vivo using animal models of cancer, such as acute lymphatic leukemia [14, 15] and primary effusion lymphoma [16]. No toxicity of HLs was observed in normal rats in vivo without any side effects [17]. Successful clinical chemotherapy with drug free HLs to patients with lymphoma has been reported after passing the committee of bioethics [18]. We have found that HLs accumulated ATL (MT-2 and MT-4) cells and inhibited the growth of ones through the induction of apoptosis via caspase-3 in vitro [19].

In this study, we investigated the therapeutic effects of HLs composed of L-α-dimyristoylphosphatidylcholine (DMPC) and polyoxymethylene(25) dodecyl ether (C₁₃EO₂₅) using model mice of ATL after the inoculation of MT-4 cells in vivo.

Hybrid liposomes (HLs) were prepared by sonication of a mixture containing 90 mol% DMPC (NOF CORPORATION, Tokyo, Japan) and 10 mol% C₁₃EO₂₅ (Nikkko Chemicals, Tokyo, Japan) in 5 % glucose solution using a bath type sonicator (VS-N300, VELVO-CLEAR, Tokyo, Japan) at 45 °C with 300 W, and filtered with a 0.20 μm cellulose acetate filter (Advantec, Tokyo, Japan). Clear solutions of HLs having a hydrodynamic diameter of 20-80 nm could be maintained for about one month. On the other hand, large and unstable
lupus having a hydrodynamic diameter of 200-1000 nm were obtained for the DMPC liposomes, which had a wide range size distribution. It is noteworthy that the HLs could avoid the reticular endothelial system in vivo [20] and thus should be appropriate for clinical applications.

First, we examined the therapeutic effects of HLs using mouse models of ATLL after the inoculation of MT-4 cells in vivo. The mice were handled in accordance with the guidelines for animal experimentation in Japanese law. The animal studies were approved by the Committee on Animal Research of Sojo University. Male Balb/c Rag-2/Jak3 double immunodeficiency mice (Balb/c-R/J mouse) [21], established by Okada et al., were employed in this study. Mice were randomly grouped on the basis of body weight by stratified randomization method. The number of mice was 6 in each group. The MT-4 cells (5.0×10^6 cells) were intrasplentically transplanted into the mice. HLs (dose: 136 mg/kg for DMPC) were intravenously administrated once each day for 14 days after one hour of the inoculation of MT-4 cells. The livers and spleens were weighed after anestomizing the mice after 56 days of inoculation of MT-4 cells, and after an autopsy the livers were fixed in 10% formalin solution, embedded in paraffin and sectioned at 5 μm of thickness. The liver sections were stained with hematoxylin and eosin (HE), and immunostained using anti-CD4 (Clone 4B12, Dako, Glostrup, Denmark) and anti-CD25 (Clone TP1/6, Abcam, Cambridge, UK) antibodies and observed by an optical microscope (ECLIPSE TS100, NIKON, Tokyo, Japan). The results are shown in Fig. 1. The liver and spleen of the groups treated with HLs (DMPC dose, 136 mg/kg) was the same as that of normal mice, although enlargements of the liver and spleen in the control and DMPC liposomes (DMPC dose, 136 mg/kg) group were confirmed. Furthermore, the relative weight of the liver and spleen in group treated with HLs was examined. Results are shown in Fig. 2. The relative organ weight of the group treated with HLs was almost the same as that of the normal group, although that of the control group without treatment obviously increased. These results suggest that HLs could inhibit the growth of ATLL cells in the liver of mouse models of ATLL.

Second, we histologically evaluated the therapeutic effects of HLs using the liver tissue for the model mice of ATLL. We examined HE staining to evaluate the therapeutic effects of HLs. The results are shown in Fig. 3 and supplementary Fig.S1. A large number of tumor cells in the control group and group treated with DMPC liposomes were observed, although few tumor cells were observed in the group treated with HLs. Moreover, we examined the immunohistochemistry to evaluate the therapeutic effects of HLs. It has been reported that CD4 and CD25 highly expressed in all stages of ATLL cells [22, 23]. Therefore, CD4 and CD25 positive cells were observed in the model mice of ATLL after the treatment with HLs. CD4 and CD25 positive cells (brown color) indicating MT-4 cells in the liver of the group treated with HLs were not observed, although numerous CD4 and CD25 positive cells were observed in the control group and the group treated with DMPC liposomes. We have already reported that HLs fused and accumulated into the cancer cells having high membrane fluidity and

Figure 1. Photographs of the liver and spleen in mouse models of ATLL treated with HLs for 14 days after the intrasplenic inoculation of MT-4 cells. Scale bar: 1cm.

![Image](image.jpg)

Figure 2. Relative organ weight of mouse models of ATLL treated with HLs for 14 days after the intrasplenic inoculation of MT-4 cells. a: Liver, b: Spleen. Data represent the mean (n=3-4) ± S.D. *: Significant difference (p < 0.05) compared with control calculated by Student’s t-test.
inhibited the growth of cancer cells [19, 24, 25]. Furthermore, it has been reported that HL accumulated ATLL (MT-2 and MT-4) cells and inhibited the growth of ones through the induction of apoptosis via caspase-3 in vitro [19]. The results suggest that HLs could be effective for inhibiting the growth of MT-4 cells in vivo.

Finally, we examined the survival ratio in mice treated with HLs. Male Balb/c-R/J mice were randomly grouped (n=4) on the basis of body weight on the day of tumor cell inoculation using the stratified randomization method. MT-4 cells (5.0×10⁵ cells) were intrasplenically injected into the Balb/c-R/J mice. HLs (dose: 68 mg/kg or 136 mg/kg for DMPC) were intravenously administered once a day for 14 days after one hour of the inoculation of tumor cells. The life prolongation rate was calculated using the following equation (life prolongation rate (%) = (survival days after the treatment) / (survival days of control group) × 100). The results are shown in Fig. 4. The median survival time of the group treated with HLs of high dose (DMPC dose, 136 mg/kg) and low dose (DMPC dose, 68 mg/kg) were 72 and 57 days, respectively. On the other hand, the median survival time for mice in the control group and in the group treated with DMPC liposomes (DMPC dose, 136 mg/kg) were 57 and 56 days, respectively. Interestingly, dose-dependent antitumor effects of HLs were obtained. Although, we have already reported the dose-dependent antitumor effects of HLs in vitro [19], it was noteworthy that a significantly prolonged survival rate of 125% (p < 0.01, analyzed using Log-rank test) was obtained in the group treated with high dose of HLs compared with control group and group treated with DMPC liposomes. These results suggest that HLs could dose-dependently inhibit the growth of MT-4 cells in vivo as well as in vitro.

With regard to the safety of HLs, no weight loss was observed in the mouse models of ATLL during the intravenous administration period of HLs for 14 days. The body weight of the mice treated with HLs was unchanged as compared with the control group (data not shown). There was no significant difference between the normal group and the group treated with HLs on the basis of gross pathology (Figs. 1 and 2) and histological analysis (Figs. 3 and S1). Furthermore, we have already reported that HLs have no side effects using normal rats in vivo [17]. These results indicate that HLs should have no severe side effects for mouse models of ATLL in vivo.

Figure 3. Micrographs of the liver in mouse models of ATLL treated with HLs after the intrasplenic inoculation of MT-4 cells using hematoxylin and eosin (HE) staining and immunostaining with anti-CD4 and anti-CD25 antibody. Dose for DMPC: 136 mg/kg. Scale bar: 1mm. Black circles indicate tumor (MT-4) cells.

Figure 4 Survival curves of model mice of ATLL treated with HLs after the intrasplenic inoculation of MT-4 cells. Data represent the mean (n=4). ◆: Control, ■: DMPC (136 mg/kg), △: HL (68 mg/kg), ○: HL (136 mg/kg).

In conclusion, the successful therapy for model mice of ATLL using HLs without any anticancer drugs was obtained. The noteworthy aspects are as follows. (1) High therapeutic effects were obtained in the mouse models of
ATLL after the treatment with HLs. (2) Few tumor cells were observed in the mouse models of ATLL treated with HLs by histological analysis. (3) Prolonged survival was obtained in the mouse models of ATLL after the treatment with HLs. These results suggest that HLs could be effective drugs for clinical application of patients with ATLL in the near future.

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