Biochemical and Biophysical Research Communications 393 (2010) 445–448

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Highly selective fusion and accumulation of hybrid liposomes into primary effusion lymphoma cells along with induction of apoptosis ☆

Tomomi Towata [a,b], Yuji Komizu [a], Shinya Suzu [b], Ryuichi Ueoka [a,*], Seiji Okada [b,*]

[a] Division of Applied Life Science, Graduate School of Engineering, Sojo University, 4-22-1 Ikeda, Kumamoto 860-0082, Japan
[b] Division of Hematopoiesis, Center for AIDS Research, Kumamoto University, 2-2-1 Honjo, Kumamoto 860-0811, Japan

ARTICLE INFO

Article history:
Received 2 February 2010
Available online 6 February 2010

Keywords:
Primary effusion lymphoma
Hybrid liposome
Membrane fluidity
Caspase-3

ABSTRACT

Primary effusion lymphoma (PEL) is an aggressive neoplasm caused by human herpes virus-8 infection, and is generally resistant to chemotherapy. Hybrid liposomes, composed of dimyristoylphosphatidylcholine (DMPC) and polyoxyethylene (21) dodecyl ether ($C_{12}(EO)_{21}$) (HL-21), were rapidly accumulated in the membrane of PEL cells. HL-21 also increased membrane fluidity of PEL cells, and induced caspase-3 activation along with cell death. These results suggest that HL-21 should be an effective and attractive regent for PEL treatment.

Introduction

Primary effusion lymphoma (PEL) has been identified as a distinct clinicopathologic entity among the non-Hodgkin's B-cell lymphomas that grow mainly in the pleural, pericardial, and abdominal cavities as a lymphomatous effusion, in the absence of an identifiable tumor mass [1–3]. PEL has been recognized as involving the infection of tumor cells by human herpes virus-8 (HHV-8) [1]. Patients are generally treated with combination chemotherapy, but durable responses are rarely seen and prognosis is poor, with median survival ranging from 2 to 6 months [2,3]. Thus, the optimal treatment for PEL has not yet been defined, and novel effective agents are to be expected.

Hybrid liposomes (HL) can be prepared by ultrasonication of a mixture of phospholipids and micellar molecules in a buffer solution, and contain no organic solvent unlike conventional liposomes [4,5]. The physiological properties of these liposomes can be controlled by changing the constituents and compositional ratios between phospholipids and micellar molecules. It has been reported that HL-21, HL-23 and HL-25 demonstrated inhibitory effects on the growth of various tumor cells *in vitro* [6–8] and *in vivo* [9,10], and remained stable for more than 3 weeks. In addition, there have been no side effects of HL in experiments using normal animals *in vivo* [9,11]. Interestingly, HL has not exerted an apoptotic effect on normal cells [12]. Thus, HL exhibit unique features as an anti-tumor drug.

We have already shown that HL induces apoptosis on PEL cells [13], but the initial step of HL to the tumor cells has not been previously analyzed. In this study, we investigated the effects of HL fusion on the PEL cells.

Materials and methods

Cell line. The human PEL cell line, BCBL-1 was obtained through the AIDS Research and Reference Reagent Program (Division of AIDS, NIAID, NIH), and were cultured in RPMI1640 medium (Wako Pure Chemical, Osaka, Japan) supplemented with 10% (v/v) heat-inactivated fetal bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin.

Preparation of hybrid liposomes (HL). HL were prepared as described [8]. Briefly, phospholipid (L-α-dimyristoylphosphatidylcholin; DMPC, Nippon Oil and fats Co. Japan) and polyoxyethylene (21) dodecyl ether ($C_{12}(EO)_{21}$, Nikko Chemicals Co., Osaka, Japan) were mixed in phosphate-buffered saline (PBS) solution and sonicated with a sonicator (VS-N300, VELVO-CLEAR, Japan) at 45 °C with 300 W followed by filtration with a 0.20 μm filter.

Accumulation of HL into the cells. HL-21 was conjugated with NBDPC as a fluorescence probe [14]. BCBL-1 cells and peripheral blood mononuclear cells (PBMC) (1×10^5 cells/ml) were treated with 100 μM HL-21/NBDPC for 30, 60, 120 and 180 min, and the

Abbreviations: PEL, primary effusion lymphoma; HL, hybrid liposomes; HHV-8, human herpes virus-8

☆ This work was supported in part by Health and Labour Science Research Grants from the Ministry of Health, Labour and Welfare of Japan (H19-AIDS-003); by the Global COE program "Global Education and Research Center Aiming at the Control of AIDS"; and Grants-in-Aid for Science Research (Nos. 20360377 and 20560732) from the Ministry of Education, Science, Sports, and Culture of Japan.

* Corresponding authors. Fax: +81 96 326 0522 (R. Ueoka), +81 96 373 6523 (S. Okada).
E-mail addresses: ueoka@life.sojo-u.ac.jp (R. Ueoka), okadas@kumamoto-u.ac.jp (S. Okada).

0006-291X/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2010.02.016

fluorescence intensity was analyzed with LSR II flow cytometry (BD Bioscience, San Jose, CA). Data were analyzed on FlowJo software (Tree Star, Ashland, OR).

Measurement of membrane fluidity. The membrane fluidity of PEL cells was measured using spectrophotofluorometer (F-4500; HITACHI) on the basis of the fluorescence depolarization method. Fluorescence depolarization (*P*) measurements of DPH can be used to study the lipid interactions or fusion of other membrane systems differing in lipid fluidity such as the interactions of viruses with cells or liposomes with cells [15]. BCBL-1 was labeled with DPH (1,6-diphenyl-1,3,5-hexatriene, Nacalai Tesque, Japan), and the *P* value of DPH was continuously measured before and after the HL treatment [12,16].

Caspase activity measurements with flowcytometry. The active caspase-3 activity was measured using PhiPhiLux-G2D2 (OncoImmunin, Gaitherssburg, MD) [17] according to the manufacturer's instructions. Briefly, BCBL-1 cells were treated with HL-21 for 5 h, incubated with 10 μM caspase substrate for 60 min, 2 μg/ml of Propidium iodide and analyzed by LSR II. Data were analyzed on FlowJo software, expressed caspase-3 positive cellular events among PI-negative (living) cells.

Results and discussion

The hybrid liposome (HL-21) was prepared as described previously [8]. Dynamic light-scattering measurements with Otsuka Electronics ELS-8000 apparatus (Japan) showed that the size of HL was less than 100 nm in diameter and remained stable for more than 1 month. It is noteworthy that HL-21 less than 100 nm in diameter could avoid the reticular endothelial system *in vivo* and thus may be appropriate for clinical applications.

At first, we examined the fusion and accumulation of HL-21 including NBDPC as a fluorescence probe [14] into the membranes of BCBL-1 cells and peripheral blood mononuclear cells (PBMC) using a flow cytometer (LSR II, BD Bioscience, San Jose, CA). Cells (1×10^5 cells/ml) were treated with 100 μM HL-21/NBDPC for 30, 60, 120 and 180 min. As shown in Fig. 1, increases in the accumulation of HL-21/NBDPC into BCBL-1 were observed in a time dependent manner. In contrast, the accumulation of HL-21/NBDPC into PBMC was low and constant. The fluorescence intensity for BCBL-1 was approximately 5-fold higher than PBMC 180 min after adding HL-21/NBDPC. These results suggest that HL-21 could selectively fuse and accumulate into PEL cells but not so much into normal lymphocytes.

Next, the membrane fluidity of PEL cells was measured using spectrophotofluorometer (F-4500; HITACHI) on the basis of the fluorescence depolarization method [15]. BCBL-1 was labeled with DPH, and the *P* value of DPH was continuously measured before and after the HL treatment [12,16]. As shown in Fig. 2, the *P* values of DPH-labeled BCBL-1 was immediately reduced with the addition of HL-21, indicating that membrane fluidity of BCBL-1 could be increased with HL, possible due to the early fusion. The result

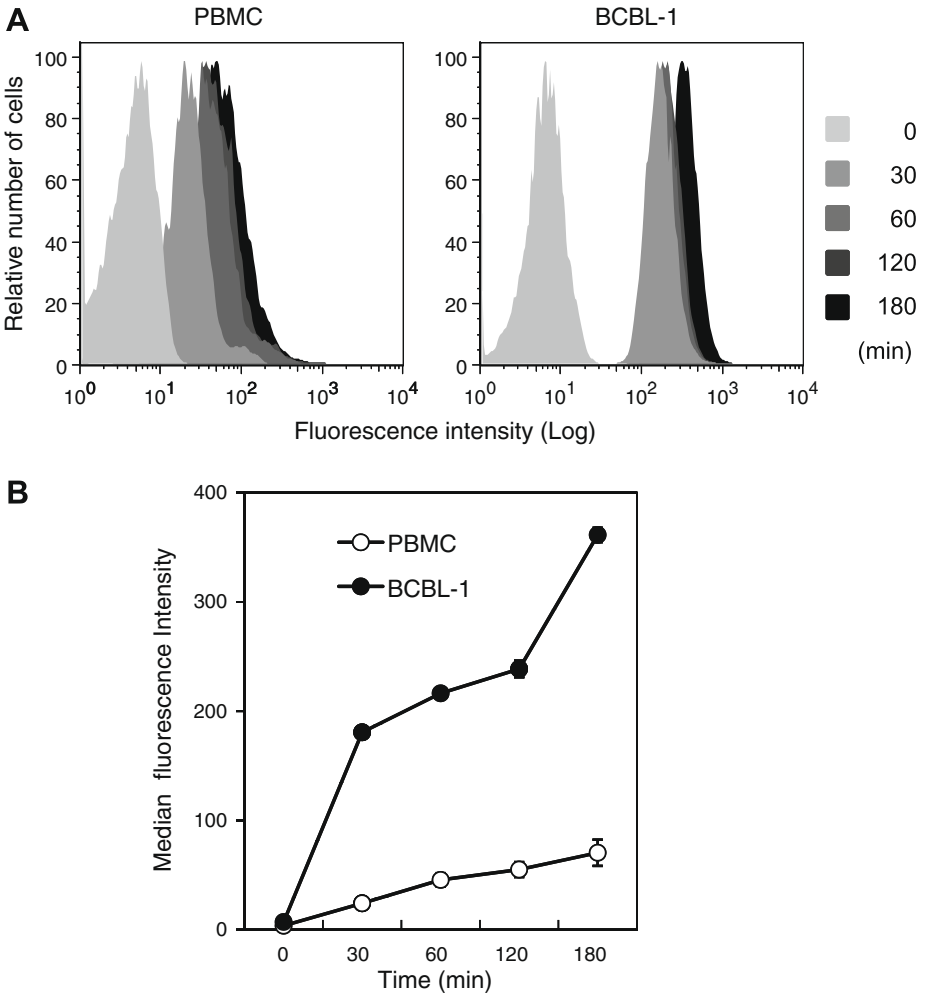

Fig. 1. Specific accumulation of HL-21 including NBDPC on PEL cells. Fluorescent intensity of NBDPC incorporated into HL-21 on PEL cells. BCBL-1 cells (1×10^5/ml) were cultured in the presence or absence of 100 μM of HL-21 including NBDPC for 30, 60, 120 and 180 min. After incubation, cells were analyzed by flow cytometry. Mean ± SD from three independent experiments.