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The antitumor effects of methyl-β-cyclodextrin against primary effusion lymphoma via the depletion of cholesterol from lipid rafts

Kumiko Gotoh [a,b,1], Ryusho Kariya [a,1], Md. Masud Alam [a], Kouki Matsuda [a], Shinichiro Hattori [a], Yuki Maeda [c], Keiichi Motoyama [c], Akihiro Kojima [b], Hidetoshi Arima [c], Seiji Okada [a,*]

[a] Division of Hematopoiesis, Center for AIDS Research, Kumamoto University, Kumamoto, Japan
[b] Radioisotope Center, Institute of Resource Development and Analysis (IRDA), Kumamoto University, Kumamoto, Japan
[c] Department of Physical Pharmaceutics, Graduate School of Pharmaceutical Sciences, Kumamoto University, Kumamoto, Japan

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ABSTRACT

Primary effusion lymphoma (PEL) is a subtype of aggressive and chemotherapy-resistant non-Hodgkin lymphoma that occurs predominantly in patients with advanced AIDS. In this study, we examined the antitumor activity of methyl-β-cyclodextrin (M-β-CyD) *in vitro* and *in vivo*. M-β-CyD quickly induced caspase-dependent apoptosis in PEL cells via cholesterol depletion from the plasma membrane. In a PEL xenograft mouse model, M-β-CyD significantly inhibited the growth and invasion of PEL cells without apparent adverse effects. These results strongly suggest that M-β-CyD has the potential to be an effective antitumor agent against PEL.

1. Introduction

Primary effusion lymphoma (PEL) is an infrequent and distinct entity of aggressive non-Hodgkin B-cell lymphoma that shows serous lymphomatous effusion in body cavities (pleura, peritoneum. and pericardium) and is universally associated with Kaposi sarcoma-associated herpes virus/human herpes virus-8 (KSHV/HHV-8) infection [1]. The lack of optimal therapy and the aggressive nature of PEL results in a short median survival of less than 6 months [2]. Therefore, there is an urgent need for the development of novel therapeutics with different antitumor mechanisms [3]. NF-κB, JAK/STAT, and phosphatidylinositol 3′-kinase (PI3K)/AKT pathways are constitutively activated and play critical roles in the survival and growth of PEL cells, and these pathways are the one of the ideal molecular targets of PEL [4–6].

The plasma membrane contains dynamic microdomains enriched in cholesterol, sphingolipids, and gangliosides termed lipid rafts [7]. Lipid rafts are integral to cellular processes by serving as organizing centers for the assembly of signaling molecules, influencing membrane fluidity and membrane trafficking, regulating neurotransmission, and receptor trafficking [8,9]. The depletion of membrane cholesterol disrupts the integrity of lipid rafts and concurrently enhances the permeability of ions and small non-electrolytes. Methyl-β-cyclodextrin (M-β-CyD), a highly water soluble cyclic heptasaccharide consisting of a β-[1–4] glucopyranose unit, has been reported as the most effective agent for the depletion of cholesterol from cells among the various cholesterol-depleting agents [10–12]. Recently, M-β-CyD by itself has been shown to have antitumor effects both *in vivo* and *in vitro* [13,14]. In this study, we examined the anti-tumor effects of M-β-CyD against PEL cells *in vitro* and *in vivo* and evaluated the potential of M-β-CyD as an antitumor agent.

2. Materials and methods

2.1. Cell lines and reagents

BCBL-1 (obtained from the NIH AIDS Reagent Program, MD) [15], BC-1 (purchased from the ATCC, Rockville, MD) [16], BC-3 (purchased from the ATCC) [17], TY-1 (a kind gift of Dr. Harutaka Katano, Institute of Infectious Diseases, Tokyo, Japan) [18], and GTO cells [19] were maintained in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum (FBS), penicillin (100 U/ml), and

Abbreviations: M-β-CyD, methyl-β-cyclodextrin; PEL, primary effusion lymphoma; PI3K, phosphatidylinositol 3′-kinase; PI, propidium iodide.

* Corresponding author at: Division of Hematopoiesis, Center for AIDS Research, Kumamoto University, 2-2-1 Honjo, Chuo-ku, Kumamoto 860-0811, Japan. Fax: +81 96 373 6523.
E-mail address: okadas@kumamoto-u.ac.jp (S. Okada).

[1] Equal contributors.

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streptomycin (100 μg/ml) in a humidified incubator at 37 °C and 5% CO_2. Methyl-β-cyclodextrin (M-β-CyD) was purchased from Sigma (St. Louis, MO).

2.2. Tetrazolium dye methylthiotetrazole (MTT) assay

The antiproliferative activities of M-β-CyD against PEL cell lines were measured by the methylthiotetrazole (MTT) method (Sigma). Briefly, 2×10^4 cells were incubated in triplicate in a 96-well microculture plate in the presence of different concentrations of M-β-CyD (0–10 mM) in a final volume of 0.1 ml for 24 h at 37 °C. Subsequently, MTT (0.5 mg/ml final concentration) was added to each well. After 3 h of additional incubation, 100 μl of a 0.04 N HCl was added to dissolve the crystals. Absorption values at 570 nm were determined with an automatic enzyme-linked immunosorbent assay (ELISA) plate reader (Multiskan; Thermo ElectronVantaa, Finland). Values were normalized to untreated (control) samples.

2.3. Cell viability assay

Cell viability was examined by the propidium iodide (PI) exclusion method as described previously [20]. Briefly, BCBL-1 cells (2×10^5 cells/ml) were cultured in the presence or absence of M-β-CyD for 1–6 h in 6-well culture plates. After being incubated, cells were stained with PI (final concentration; 2 μg/ml) and cell viability was analyzed by LSR II flow cytometry (BD Bioscience, San Jose, CA). Data were analyzed with FlowJo software (Tree Star, San Carlos, CA).

2.4. Determination of cholesterol in the culture medium

BCBL-1 and GTO cells (1×10^6 cells/35 mm dish) were incubated with 10 mM M-β-CyD in RPMI-1640 culture medium (Phenol Red-free) at 37 °C for 1 h. After centrifugation (10,000 rpm, 5 min) of the culture medium, the supernatant was recovered. Total cholesterol in the culture medium was determined using Cholesterol-test Wako® (Wako Pure Chemical Industries, Osaka, Japan).

2.5. Western blot analysis

BCBL-1 cells with or without the treatment were collected and washed in cold PBS before the addition of NP40 buffer (50 mM Tris–HCl, pH 7.4, 150 mM NaCl, 1 % NP-40, 1 mM NaF, 1 mM Na_3VO_4, and protease inhibitor cocktail). After being incubated on ice for 1 h, the samples were centrifuged at 15,000 rpm for 15 min and the supernatant was collected as a whole cell lysate. Proteins (20 μg protein) were separated by SDS–PAGE and blotted onto a PVDF membrane (GE Healthcare, Buckinghamshire, UK). The primary antibodies used were as follows: anti-caspase9 (4502), anti-cleaved caspase8 (9661), anti-caspase3 (9496), anti-Akt (4691), anti-phospho (Thr308)-Akt (2965) (Cell Signaling Technology, Danvers, MA), and anti-actin (C-2) (Santa Cruz Biotechnology, Santa Cruz, CA). Detection was performed using Chemi-Lumi One Super reagents (Nacalai Tesque, Kyoto, Japan). Western blots were quantified using the ImageQuant LAS 4000 system (GE Healthcare). Relative density was evaluated and normalized with actin.

2.6. Xenograft mouse model

NOD/Rag-2/Jak3-double deficient (NRJ) mice [21] were housed and monitored in our animal research facility according to institutional guidelines. All experimental procedures and protocols were approved by the Institutional Animal Care and Use Committee at Kumamoto University. Eight- to ten-week-old female NRJ mice were intraperitoneally inoculated with 7×10^6 BCBL-1 cells suspended in 200 μl PBS. The mice were then treated with intraperitoneal injections of PBS or M-β-CyD (500 mg/kg per day). Tumor burdens were evaluated by measuring body weights and ascites.

2.7. Immunohistochemistry

To investigate the expression of the KSHV/HHV-8 ORF73 (LANA) protein, tissue samples were fixed with 10% neutral-buffered formalin, embedded in paraffin and cut into 4-μm sections. The sections were deparaffinized by sequential immersions in xylene and ethanol, and rehydrated in distilled water. They were then irradiated for 15 min in a microwave oven for antigen retrieval. Endogenous peroxidase activity was blocked by immersing the sections in methanol/0.6% H_2O_2 for 30 min at room temperature. Rat MAb to KSHV/HHV-8 ORF73 (Advanced Biotechnologies, Columbia, MD), diluted 1:1000 in PBS/5% bovine serum albumin (BSA), was then applied, and the sections were incubated overnight at 4 °C. After washing in PBS twice, polyclonal rabbit anti-Rat Ig-HRP (DAKO, Copenhagen, Denmark) were applied according to the manufacturer's instructions. The signal was visualized using the Histofine SAB-PO(M) kit (Nichirei Bioscience, Tokyo, Japan).

2.8. Blood count and serum LDH

Red blood cell counts and hemoglobin concentrations were measured using the Sysmex F-520 hematology analyzer (Sysmex Corp., Kobe, Japan). Serum LDH was measured using the Cytotoxicity Detection Kit PLUS (LDH) (Roche, Penzberg, Germany) according to manufacturer's instructions.

2.9. Statistical analysis

All assays were performed in triplicate and expressed as mean values ± SD. The significance of differences observed between experimental groups was determined using the Student's *t*-test. *P* values less than 0.05 were considered significant.

3. Results

3.1. Inhibitory effects of M-β-CyD on the growth of PEL cell lines

First, we examined the effects of M-β-CyD on the growth of five PEL cell lines (BCBL-1, BC-1, BC-3, TY-1, and GTO). PEL cell lines were cultured with various doses of M-β-CyD for 24 h, and MTT assays were performed. As shown in Fig. 1, M-β-CyD inhibited the growth of all PEL cell lines in a dose-dependent manner. The

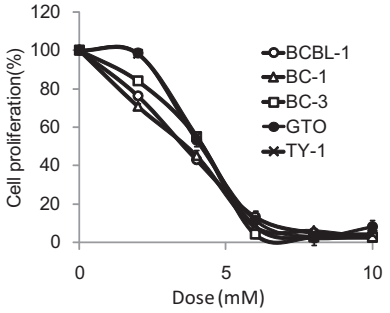

Fig. 1. Cytotoxic activity of M-β-CyD in PEL cells. Five PEL cell lines (BCBL-1, BC-1, BC-3, TY-1, and GTO) were incubated with various doses of M-β-CyD for 24 h, and the MTT assay was performed. One representative result from 3 independent experiments is shown.

half maximal inhibitory concentration (IC_{50}) was 3.33–4.23 mM in each cell line.

3.2. Induction of apoptosis in PEL cells by M-β-CyD

Next, we stained PEL cells with propidium iodide (PI) to determine cell death. As shown in Fig. 2A and B, the number of PI-positive cells increased in time- and dose-dependent manners. So, we confirmed the induction of apoptosis by Western blotting. Cleaved caspases were detected as early as 3 h after the stimulation with M-β-CyD (Fig. 2C), indicating that M-β-CyD induced apoptosis in PEL cell lines.

3.3. Effect of cholesterol extraction of M-β-CyD

Since we previously reported that M-β-CyD induced apoptosis through cholesterol depletion in tumor cells, we investigated the effects of M-β-CyD on the release of cholesterol from PEL cells to the culture medium. As shown in Fig. 3A, cholesterol released in the culture medium after the incubation with 10 mM M-β-CyD for 1 h was determined for both the BCBL-1 and GTO cell lines. Next, to confirm the involvement of cholesterol depletion in apoptosis induced by M-β-CyD, we examined the induction of cell death and cleaved caspase 3 in BCBL-1 cells in the cholesterol-loaded medium containing M-β-CyD. As shown in Fig. 3B, cell death was induced with M-β-CyD in a dose-dependent manner, and the cytotoxic activity of M-β-CyD in BCBL-1 cells was significantly lower in the saturated cholesterol-loading culture. The induction of cleaved caspase 3 was also significantly suppressed in the saturated cholesterol-loading culture medium (Fig. 3C). Taken together, these results suggested that M-β-CyD induced apoptosis in PEL cells through the extraction of cholesterol from the plasma membrane.

Since 2, 6-di-*O*-mechyl-β-cyclodextrin (DM-β-CyD), another type of frequently used cyclodextrin, was previously shown to inhibit the phosphorylation of Akt through the extraction of cholesterol from the plasma membrane [22], we analyzed the expression of Akt and phosphorylated Akt by M-β-CyD. Although the phosphorylation of Akt was markedly suppressed by the addition of M-β-CyD, the total amount of Akt was not changed.

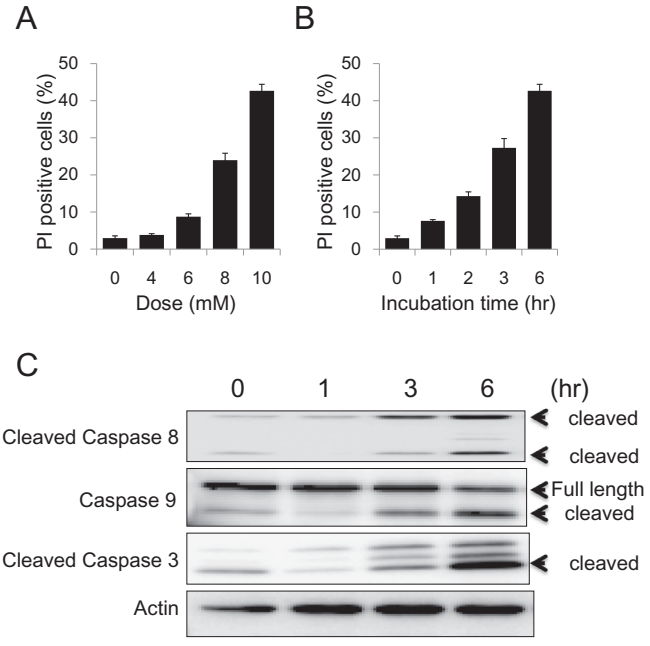

Fig. 2. Induction of apoptosis by M-β-CyD in PEL cells. (A) BCBL-1 cells were incubated with various doses of M-β-CyD for 6 h, PI was added to detect dead cells and analyzed with flow cytometry. (B) BCBL-1 cells were incubated with 10 mM of M-β-CyD for 0–6 h, PI was added to detect dead cells and analyzed with flow cytometry. (C) BCBL-1 cells were incubated with 10 mM of M-β-CyD for 0–6 h, and cleaved caspase 8, 9, 3 were detected with Western blot analysis. One representative result from 3 independent experiments is shown.

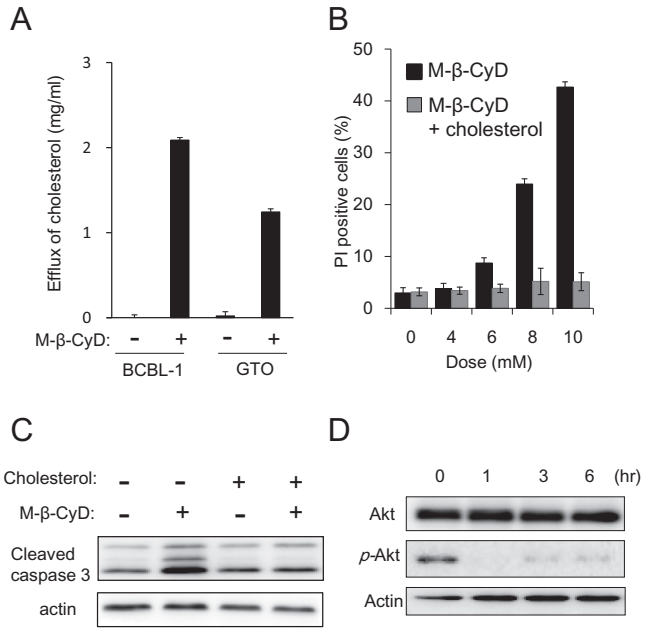

Fig. 3. M-β-CyD eluted cholesterol from the plasma membrane and induced apoptosis via the suppression of Akt. (A) Efflux of cholesterol from lipid rafts to the culture medium after the treatment of BCBL-1 and GTO cells with M-β-CyD (10 mM). (B) Effect of cholesterol on the cytotoxic activity of M-β-CyD (10 mM) in BCBL-1 cells. (C) Effect of cholesterol on the activation of caspase 3. (D) Suppression of phosphorylated Akt (*p*-Akt) with the M-β-CyD treatment. BCBL-1 cells were incubated with 10 mM of M-β-CyD for 0–6 h, and total and phosphorylated Akt were detected using Western blot analysis. One representative result from 3 independent experiments is shown.

3.4. In vivo effects of M-β-CyD on severe immunodeficient mice inoculated with BCBL-1 cells

Since the above results suggested the efficacy of M-β-CyD for the treatment of PEL patients, we next examined the *in vivo* effects of M-β-CyD in a xenografted mouse model. NOD/Rag-2/Jak3-deficient (NRJ) mice were inoculated intraperitoneally with 7×10^6 BCBL-1 cells, which is a clinically relevant PEL model [23]. A dose of 500 mg/kg of M-β-CyD was administrated via an intraperitoneal injection on day 3 after the BCBL-1 cell inoculation and everyday thereafter for 21 days by the schedule described previously [23]. M-β-CyD-treated mice appeared to be healthy, whereas non-treated mice had a distended abdominal region (Fig. 4A). The body weights of non-treated mice were significantly higher than those of M-β-CyD treated mice (32.2 ± 2.0 g versus 28.3 ± 1.4 g, $n = 7$, $p < 0.01$) probably due to the retention of ascites. As shown in Fig. 4B, M-β-CyD-treated mice had a significantly lower volume of ascites (0.13 ± 0.19 ml) than that of non-treated mice (2.34 ± 0.49 ml) ($p < 0.01$). In addition, lung invasion by PEL cells was evaluated by LANA staining, and revealed that the number of LANA-positive cells in M-β-CyD-treated mice was significantly lower than that in untreated mice (17.6 ± 7.6 cells versus 159.0 ± 20.5 cells per field magnification, $\times 100$, $n = 5$, $p < 0.001$, Fig. 4D). These results indicated that M-β-CyD significantly inhibited the growth of PEL cells *in vivo*.

Since a systemic injection of M-β-CyD is known to induce hemolysis, we examined the RBC count, Hb, and LDH of

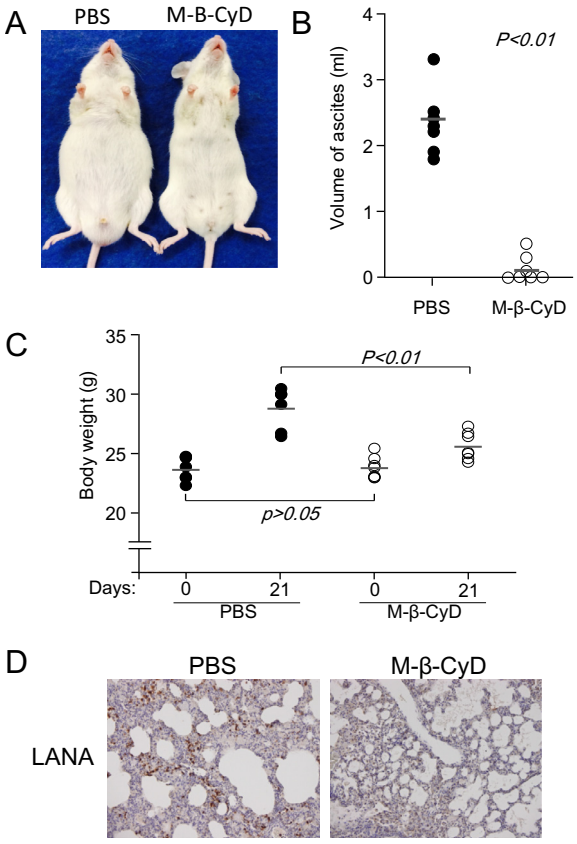

Fig. 4. Treatment of NRJ mice with M-β-CyD inhibited the growth of PEL *in vivo*. (A) A photograph of untreated and M-β-CyD mice 3 weeks after being inoculated intraperitoneally with BCBL-1 cells. (B) The body weights of mice inoculated with BCBL-1 cells, untreated mice, and M-β-CyD-treated mice. Shown as the mean ± SD of 7 mice ($p < 0.01$). (C) The volume of ascites in mice inoculated with BCBL-1 cells, untreated mice, and M-β-CyD-treated mice. Shown as the mean ± SD of 7 mice ($p < 0.001$). (D) Metastasis of PEL cells into the lungs of BCBL-1-inoculated NRJ mice. Immunohistochemical staining using was performed anti-LANA to detect BCBL-1.

M-β-CyD-treated mice. As shown in Table 1, the M-β-CyD treatment at this dose did not induce hemolysis, indicating that a local injection of M-β-CyD into the peritoneal cavity had no severe adverse effects *in vivo*.

4. Discussion

In the present study, we revealed that M-β-CyD potentially caused apoptosis in PEL cells by depleting cholesterol from lipid rafts in the plasma membrane, and M-β-CyD markedly inhibited tumor growth after a peritoneal injection into PEL-bearing mice.

Phosphatidylinositol 3′-kinase (PI3K) activates Akt through the recruitment of Akt and 3-phospho-inositide-dependent protein kinase-1 (PDK1) to lipid rafts [4,24]. Activated Akt prevents apoptosis by generating anti-apoptotic signals through the phosphorylation of Bad, GSK3, and caspase-9 as well as the activation of transcription factors such as Forkhead and NF-κB [25]. In the case of PEL, the K1 protein of KSHV/HHV-8 has been shown to activate PI3K [26] while the G protein-coupled receptor (vGPCR) of KSHV/HHV-8 transformed cells by targeting Akt [27]. Motoyama et al. previously showed that 2,6-di-*O*-mechyl-β-cyclodextrin (DM-β-CyD) significantly suppressed the phosphorylation of Akt and accelerated the degradation of Akt through the extraction of cholesterol from lipid rafts [22]. In the present study, we also showed that cholesterol was extracted from lipid rafts and the phosphorylation of Akt was completely suppressed with M-β-CyD (Fig. 3D) in PEL cells. Thus, M-β-CyD could suppress the activation of the PI3K/Akt pathway and induce apoptosis, indicating that M-β-CyD is a potential reagent for the treatment of PEL.

M-β-CyD exhibited *in vivo* antitumor effects after a peritoneal injection to PEL-bearing mice without apparent adverse effects (Fig. 4 and Table1). Although M-β-CyD has been shown to have strong antitumor effects following a direct intratumoral injection [13], we have previously shown that the intravenous administration of M-β-CyD did not have any antitumor effects, probably due to the lack of target specificity against tumor cells and rapid renal clearance from the body [14]. In addition, the intravenous administration of M-β-CyD was shown to significantly change blood chemistry values, especially LDH and BUN, suggesting the induction of hemolysis by M-β-CyD. Thus, the clinical application of M-β-CyD for systemic chemotherapy requires M-β-CyD to be modified so that it only targets tumor cells. Using this strategy, Onodera et al. recently succeeded in targeting folate receptor-expressing tumors by folate-appended M-β-CyD without systemic adverse effects [28,29]. On the other hand, since PEL usually shows serous lymphomatous effusion in body cavities (pleura, peritoneum, and pericardium), a local injection of M-β-CyD was effective in PEL-bearing mice. Hemolysis, one of the main adverse effects of M-β-CyD, did not occur in spite of the daily administration of a high dose of M-β-CyD (Table 1), which can explain why M-β-CyD remained in the peritoneal cavity and gradually moved into the veins. Taken together, M-β-CyD is a promising candidate against this aggressive and chemotherapy-resistant lymphoma.

Cyclodextrins (CyDs) including M-β-CyD have been used as the base of pharmaceutical reagents because they can improve the solubility, bioavailability, delivery, and stability of drugs. Several groups have shown that M-β-CyD and its modified forms enhanced the cytotoxic effect of various chemotherapeutic drugs [28,30–32]. In this study, we showed that M-β-CyD alone was effective for the treatment of PEL, indicating the potential synergistic effects of chemotherapeutic drugs with M-β-CyD. In addition, the modification of M-β-CyD may potentiate the targeting of cancer cells and reduce systemic adverse effects [28]. Thus, M-β-CyD in combination with chemotherapeutic drugs provides a unique therapeutic window for improvements in the treatment of this aggressive and drug-resistant lymphoma.

In conclusion, we demonstrated here that M-β-CyD had potent anti-PEL activity *in vivo* without apparent adverse effects including hemolysis. Since M-β-CyD has unique functional properties, it is a promising candidate for novel chemotherapy against PEL.

Table 1
Hematological findings of M-β-CyD treated mice.

Formulation	RBC ($\times 10^4$/mm^3)	Hemoglobin (g/dl)	LDH (U/L)[a]
Control mice	763.7 ± 60.8	12.1 ± 3.0	197.8 ± 20.7
PEL-inoculated untreated mice	707.6 ± 123.7	11.9 ± 3.3	205.7 ± 15.3
PEL-inoculated M-β-CyD-treated mice	726.1 ± 132.2	11.8 ± 2.3	198.5 ± 18.9

Each value represents the mean ± SD of 6–7 mice.
[a] LDH: lactate dehydrogenase.

Conflict of interest

The authors declare no competing financial interests.

Contributions

K.G., R.K., and S.O. designed the research; K.G., R.K., M.M.A., K.M., A.K., and H.A. performed the research; K.G., R.K., and S.O. wrote the manuscript.

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References

[1] Y.B. Chen, A. Rahemtullah, E. Hochberg, Primary effusion lymphoma, Oncologist 12 (2007) 569–576.
[2] E. Boulanger, L. Gerard, J. Gabarre, J.M. Molina, C. Rapp, J.F. Abino, J. Cadranel, S. Chevret, E. Oksenhendler, Prognostic factors and outcome of human herpesvirus 8-associated primary effusion lymphoma in patients with AIDS, J. Clin. Oncol. 23 (2005) 4372–4380.
[3] H. Goto, S. Okada, New approaches to treating primary effusion lymphoma, Expert. Opin. Orphan Drugs 1 (2013) 1019–1029.
[4] S. Uddin, A.R. Hussain, K.A. Al-Hussein, P.S. Manogaran, A. Wickrema, M.I. Gutierrez, K.G. Bhatia, Inhibition of phosphatidylinositol 3′-kinase/AKT signaling promotes apoptosis of primary effusion lymphoma cells, Clin. Cancer Res. 11 (2005) 3102–3108.
[5] Y. Aoki, G.M. Feldman, G. Tosato, Inhibition of STAT3 signaling induces apoptosis and decreases survivin expression in primary effusion lymphoma, Blood 101 (2003) 1535–1542.
[6] S.A. Keller, E.J. Schattner, E. Cesarman, Inhibition of NF-kappaB induces apoptosis of KSHV-infected primary effusion lymphoma cells, Blood 96 (2000) 2537–2542.
[7] K. Simons, R. Ehehalt, Cholesterol, lipid rafts, and disease, J. Clin. Invest. 110 (2002) 597–603.
[8] L.J. Pike, The challenge of lipid rafts, J. Lipid Res. 50 (Suppl.) (2009) S323–S328.
[9] K. Simons, J.L. Sampaio, Membrane organization and lipid rafts, Cold Spring. Harbor Perspect. Biol. 3 (2011) a004697.
[10] J. Pitha, T. Irie, P.B. Sklar, J.S. Nye, Drug solubilizers to aid pharmacologists: amorphous cyclodextrin derivatives, Life Sci. 43 (1988) 493–502.
[11] E.P. Kilsdonk, P.G. Yancey, G.W. Stoudt, F.W. Bangerter, W.J. Johnson, M.C. Phillips, G.H. Rothblat, Cellular cholesterol efflux mediated by cyclodextrins, J. Biol. Chem. 270 (1995) 17250–17256.
[12] P.G. Yancey, W.V. Rodrigueza, E.P. Kilsdonk, G.W. Stoudt, W.J. Johnson, M.C. Phillips, G.H. Rothblat, Cellular cholesterol efflux mediated by cyclodextrins. Demonstration of kinetic pools and mechanism of efflux, J. Biol. Chem. 271 (1996) 16026–16034.
[13] P.Y. Grosse, F. Bressolle, F. Pinguet, Antiproliferative effect of methyl-beta-cyclodextrin in vitro and in human tumour xenografted athymic nude mice, Br. J. Cancer 78 (1998) 1165–1169.
[14] R. Onodera, K. Motoyama, A. Okamatsu, T. Higashi, R. Kariya, S. Okada, H. Arima, Involvement of cholesterol depletion from lipid rafts in apoptosis induced by methyl-beta-cyclodextrin, Int. J. Pharm. 452 (2013) 116–123.
[15] R. Renne, W. Zhong, B. Herndier, M. McGrath, N. Abbey, D. Kedes, D. Ganem, Lytic growth of Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8) in culture, Nat. Med. 2 (1996) 342–346.
[16] E. Cesarman, P.S. Moore, P.H. Rao, G. Inghirami, D.M. Knowles, Y. Chang, In vitro establishment and characterization of two acquired immunodeficiency syndrome-related lymphoma cell lines (BC-1 and BC-2) containing Kaposi's sarcoma-associated herpesvirus-like (KSHV) DNA sequences, Blood 86 (1995) 2708–2714.
[17] L. Arvanitakis, E.A. Mesri, R.G. Nador, J.W. Said, A.S. Asch, D.M. Knowles, E. Cesarman, Establishment and characterization of a primary effusion (body cavity-based) lymphoma cell line (BC-3) harboring kaposi's sarcoma-associated herpesvirus (KSHV/HHV-8) in the absence of Epstein-Barr virus, Blood 88 (1996) 2648–2654.
[18] H. Katano, Y. Hoshino, Y. Morishita, T. Nakamura, H. Satoh, A. Iwamoto, B. Herndier, S. Mori, Establishing and characterizing a CD30-positive cell line harboring HHV-8 from a primary effusion lymphoma, J. Med. Virol. 58 (1999) 394–401.
[19] H. Goto, Y. Kojima, H. Nagai, S. Okada, Establishment of a CD4-positive cell line from an AIDS-related primary effusion lymphoma, Int. J. Hematol. 97 (2013) 624–633.
[20] A.R. Gottschalk, L.H. Boise, C.B. Thompson, J. Quintans, Identification of immunosuppressant-induced apoptosis in a murine B-cell line and its prevention by Bcl-x but not Bcl-2, Proc. Natl. Acad. Sci. U.S.A. 91 (1994) 7350–7354.
[21] H. Goto, R. Kariya, M. Shimamoto, E. Kudo, M. Taura, H. Katano, S. Okada, Antitumor effect of berberine against primary effusion lymphoma via inhibition of NF-kappaB pathway, Cancer Sci. 103 (2012) 775–781.
[22] K. Motoyama, K. Kameyama, R. Onodera, N. Araki, F. Hirayama, K. Uekama, H. Arima, Involvement of PI3K-Akt-Bad pathway in apoptosis induced by 2,6-di-O-methyl-beta-cyclodextrin, not 2,6-di-O-methyl-alpha-cyclodextrin, through cholesterol depletion from lipid rafts on plasma membranes in cells, Eur. J. Pharm. Sci. 38 (2009) 249–261.
[23] N. Takahashi-Makise, S. Suzu, M. Hiyoshi, T. Ohsugi, H. Katano, K. Umezawa, S. Okada, Biscoclaurine alkaloid cepharanthine inhibits the growth of primary effusion lymphoma in vitro and in vivo and induces apoptosis via suppression of the NF-kappaB pathway, J. Int. Cancer 125 (2009) 1464–1472.
[24] X. Gao, P.R. Lowry, X. Zhou, C. Depry, Z. Wei, G.W. Wong, J. Zhang, PI3K/Akt signaling requires spatial compartmentalization in plasma membrane microdomains, Proc. Natl. Acad. Sci. U.S.A. 108 (2011) 14509–14514.
[25] S.R. Datta, A. Brunet, M.E. Greenberg, Cellular survival: a play in three Akts, Genes Dev. 13 (1999) 2905–2927.
[26] O. Prakash, Z.Y. Tang, X. Peng, R. Coleman, J. Gill, G. Farr, F. Samaniego, Tumorigenesis and aberrant signaling in transgenic mice expressing the human herpesvirus-8 K1 gene, J. Natl Cancer Inst. 94 (2002) 926–935.
[27] S. Montaner, Akt/TSC/mTOR activation by the KSHV G protein-coupled receptor: emerging insights into the molecular oncogenesis and treatment of Kaposi's sarcoma, Cell Cycle 6 (2007) 438–443.
[28] R. Onodera, K. Motoyama, A. Okamatsu, T. Higashi, H. Arima, Potential use of folate-appended methyl-beta-cyclodextrin as an anticancer agent, Sci. Rep. 3 (2013) 1104.
[29] R. Onodera, K. Motoyama, N. Tanaka, A. Ohyama, A. Okamatsu, T. Higashi, R. Kariya, S. Okada, H. Arima, Involvement of autophagy in antitumor activity of folate-appended methyl-beta-cyclodextrin, Sci. Rep. 4 (2014) 4417.
[30] A.K. Upadhyay, S. Singh, R.R. Chhipa, M.V. Vijayakumar, A.K. Ajay, M.K. Bhat, Methyl-beta-cyclodextrin enhances the susceptibility of human breast cancer cells to carboplatin and 5-fluorouracil: involvement of Akt, NF-kappaB and Bcl-2, Toxicol. Appl. Pharmacol. 216 (2006) 177–185.
[31] N. Mohammad, P. Malvi, A.S. Meena, S.V. Singh, B. Chaube, G. Vannuruswamy, M.J. Kulkarni, M.K. Bhat, Cholesterol depletion by methyl-beta-cyclodextrin augments tamoxifen induced cell death by enhancing its uptake in melanoma, Mol. Cancer 13 (2014) 204.
[32] N. Rocks, S. Bekaert, I. Coia, G. Paulissen, M. Gueders, B. Evrard, J.C. Van Heugen, P. Chiap, J.M. Foidart, A. Noel, D. Cataldo, Curcumin-cyclodextrin complexes potentiate gemcitabine effects in an orthotopic mouse model of lung cancer, Br. J. Cancer 107 (2012) 1083–1092.