Bioorganic & Medicinal Chemistry Letters 18 (2008) 4578–4580

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Selective inhibitory effects of hybrid liposomes on the growth of HIV type 1-infected cells in vitro

Ryuichi Ueoka [a,*], Yuji Komizu [a], Yoko Matsumoto [a], Yu Zhong [b], Ritsuko Tanaka [b], Naoki Yamamoto [b,c,*]

[a] Graduate Course of Applied Life Science, Sojo University, 4-22-1 Ikeda, Kumamoto 860-0082, Japan
[b] Department of Molecular Virology, Tokyo Medical and Dental University School of Medicine, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8519, Japan
[c] AIDS Research Center, National Institute of Infectious Diseases, 1-23-1 Toyama, Shinjuku-ku, Tokyo 162-8640, Japan

ARTICLE INFO

Article history:
Received 14 May 2008
Revised 8 July 2008
Accepted 10 July 2008
Available online 15 July 2008

Keywords:
Liposome
HIV-1
Membrane fluidity
Latency
Growth inhibition
Virus production

ABSTRACT

A good correlation between fifty-percent inhibitory concentration (IC_{50}) of hybrid liposomes (HL) composed of dimyristoylphosphatidylcholine and polyoxyethylene(*n*) dodecyl ether on the growth of MOLT-4/IIIB cells (MOLT-4 cells chronically infected with human immunodeficiency virus (HIV)) and the membrane fluidity of HL was obtained. Furthermore, the huge enhancement of virus production was observed in the latently HIV-infected (J_{22}-HL-60) cells after the treatment with HL.

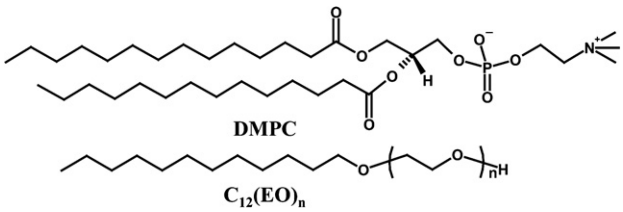
© 2008 Elsevier Ltd. All rights reserved.

It is well known that latently infected cells are activated by cytokines, lipopolysaccharides, monoclonal antibodies, and other factors, which enhance viral replication and antiviral immune responses.[1,2] Highly active antiretroviral therapy (HAART) has succeeded in lowering HIV levels in most patients, in some cases to undetectable levels. However, HAART alone cannot completely eradicate the virus for the following reason. That is, the latent HIV-1 persists in resting $CD4^+$ T-lymphocyte and monocyte-macrophage as potential source of reactivation of viral replication, even in patients receiving the therapy.[3,4] So, new approaches for the elimination of these chronically infected cells are urgently needed.

On the other hand, hybrid liposomes (HL), first developed by Ueoka et al. (Scheme 1),[5] can be prepared by simply ultrasonicating a mixture of vesicular and micellar molecules in a buffer solution. HL composed of dimyristoylphosphatidylcholine (DMPC) and polyoxyethylenealkyl ether have remarkable inhibitory effects on the growth of various tumor cells along with apoptosis in vitro and in vivo.[6–8] Furthermore, successful clinical chemotherapy with drug-free HL to patients with lymphoma has been reported.[7,9] We also reported that HL distinguished between normal and tumor cells, and then fused and accumulated into the membranes of tumor cells leading to apoptosis.[10,11]

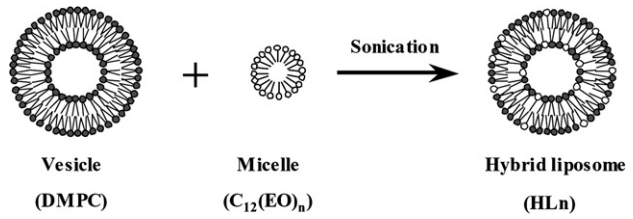
In this study, we investigated the inhibitory effects of hybrid liposomes composed of DMPC and polyoxyethylene(n)dodecyl ether ($C_{12}(EO)_n$; $n = 4$, 8, 10, 21, 23, and 25) on the growth of HIV-1 chronically infected cells in vitro.

The hybrid liposomes (HL-*n*) were prepared by sonication of a mixture containing 90 mol% DMPC and 10 mol% $C_{12}(EO)_n$ in phosphate-buffered saline (Ca^{2+}, Mg^{2+} free) solution at 45 °C with 300 W as described previously.[8] The sample solutions were sterilized using a membrane filter with a 0.20 μm pore size. Morphology of HL-*n* was examined on the basis of dynamic light scattering measurements. We found the size of HL-*n* ($n = 21$, 23, and 25) was less than 100 nm diameter and stable for more than 1 month. On the other hand, it was found that DMPC liposomes and HL-*n* ($n = 4$, 8, and 10) were unstable. It is worthy to note that HL-*n* ($n = 21$, 23, 25) having less than 100 nm in diameter could avoid the reticular endothelial system in vivo[12] and should be appropriate for clinical applications.[7,9]



* Corresponding authors. Tel.: +81 96 326 3952; fax: +81 96 326 0522 (R.U.).
E-mail address: ueoka@life.sojo-u.ac.jp (R. Ueoka).

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.07.030



Scheme 1. Schematic representation of hybrid liposomes.

Firstly, we examined the 50% inhibitory effects (IC_{50}) of HL-*n* on the growth of MOLT-4 and MOLT-4/IIIB cells on the basis of MTT assay.[13] MOLT-4/IIIB cells were MOLT-4 cells chronically infected with HIV-1 (HTLV-IIIB strain).[14] MOLT-4 cells were derived from a human T cell leukemia line. These cells (3.0×10^5 viable cells/ml) were cultured for 5 days in a humidified 5% CO_2 incubator at 37 °C after adding the sample solutions.[15] The MTT solution was added to the cells and the absorbance at a wavelength of 450 nm was measured by spectrophotometer. Cell viability was estimated by $(A_{Mean}/A_{Control}) \times 100$, where A_{Mean} and $A_{Control}$ denote the absorbance of water-insoluble formazan, in the presence and absence of sample solutions, respectively. IC_{50} was determined by the concentration dependence of viability. The results are summarized in Table 1. The results reveal that some liposomes had selective inhibitory effects on the growth of virus-infected MOLT-4 cells. The IC_{50} values of HL-25 for MOLT-4 and MOLT-4/IIIB cells were 221 and 40 μM, respectively (Fig. 1). The selectivity index (*SI*) is based on the ratio of the IC_{50} for infected cells to the IC_{50} for uninfected cells. It is attractive that the high *SI* values were obtained for DMPC liposomes (5.9) and HL-25 (5.7). On the other hand, the moderate *SI* values were obtained in the case of HL-4 (3.2), HL-21 (3.6), and HL-23 (4.1). And, the small *SI* values were obtained for HL-8 (2.0) and HL-10 (1.6). These results indicate that DMPC liposomes and HL-25 might be specific for inhibiting the growth of HIV-1 chronically infected cells. Previously, Aloia et al. reported that cholesterol-to-phospholipid molar ratio was found to be lower in plasma membrane isolated from HIV-1-infected cells compared with uninfected cells in vitro,[16] similar to that observed for normal and tumor cells.[17,18] It is suggested that the fluidity of infected cell membranes as molecular aggregates could be larger than that of uninfected cells. We have already reported that tumor cell membrane should be targeted by HL leading to growth inhibition and apoptosis.[10,11] So, the inhibitory effects of HL in this study might be related to differences between biophysical properties of plasma membranes of uninfected and infected MOLT-4 cells.

How do HL suppress the growth of HIV-infected cells?

We evaluated the membrane fluidity of HL on the basis of fluorescence depolarization (*P*) of 1, 6-diphenyl-1, 3, 5-hexatriene (DPH).[19] The *P* value for DPH is caused by the molecular motion of the fluorescence probe, which reflects the microviscosity of the surrounding region.[20] The results are shown in Figure 2. It is worthy to note that a good correlation between the *P* values in HL and the IC_{50} values on the growth of MOLT-4/IIIB cells was obtained. This indicates that the fluidity of HL should be correlated directly with the growth inhibition of HIV-1 chronically infected cells. We have already reported that a good correlation between IC_{50} of HL-*n* (*n* = 4, 8, 10, 21, 23, and 25) on the growth of human colorectal tumor (WiDr) cells and the membrane fluidity of HL was obtained.[11] These results suggest that the growth inhibition of HL for HIV-1 infected MOLT-4 cells as well as tumor cells could be related to the fluidity of HL membrane.

Similar selective cytotoxicity against HIV-1-infected cells was observed in several agents such as tumor necrosis factor (TNF) alpha,[1,21] Fas (CD95) ligand,[21] OX40 (CD134) ligand,[22] phorbol esters,[23,24] intracellular calcium pump inhibitors,[25] and ascorbic acid,[26] and these factors also induced virus from latently in-

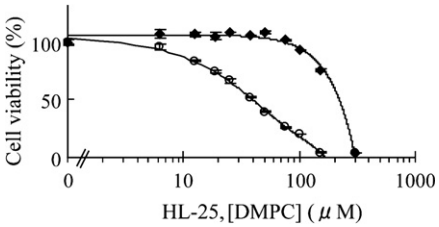


Figure 1. Cell viability of MOLT-4 and MOLT-4/IIIB cells after the treatment with HL-25. MOLT-4 (closed rhombus), MOLT-4/IIIB (open circle). The concentration ranges were [DMPC] = 6.3–300 μM and [$C_{12}(EO)_{25}$] = 0.7–33 μM for DMPC and $C_{12}(EO)_{25}$, respectively. Data represents means ± SD.

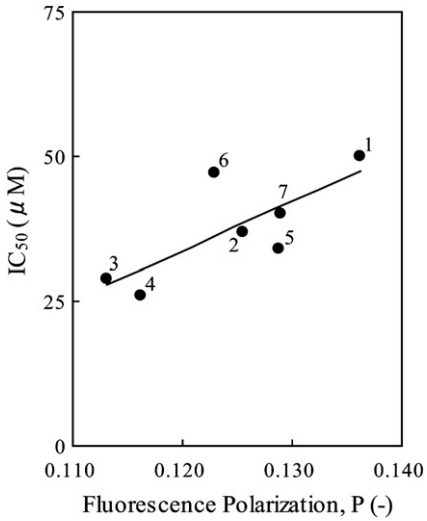


Figure 2. The relationship between *P* of DPH in HL and IC_{50} for MOLT-4/IIIB cells. 1, DMPC; 2, HL-4; 3, HL-8; 4, HL-10; 5, HL-21; 6, HL-23; 7, HL-25. Statistical analysis ($R = 0.76$, $p = 0.047$).

Table 1
Inhibitory effects of HL-*n* on the growth of MOLT-4 and MOLT-4/IIIB cells

Liposomes	MOLT-4 IC_{50}, [DMPC] μM[a]	MOLT-4/IIIB IC_{50}, [DMPC] μM[a]	SI[b]
DMPC	293	50	5.9
HL-4	118	37	3.2
HL-8	59	29	2.0
HL-10	41	26	1.6
HL-21	121	34	3.6
HL-23	190	47	4.1
HL-25	221	40	5.6

[a] IC_{50} was defined as the concentration of HL required to reduce the viability and proliferation of cells by 50%.
[b] Selectivity index (SI), based on the ratio of the IC_{50} for virus-infected cells to the IC_{50} for uninfected cells.

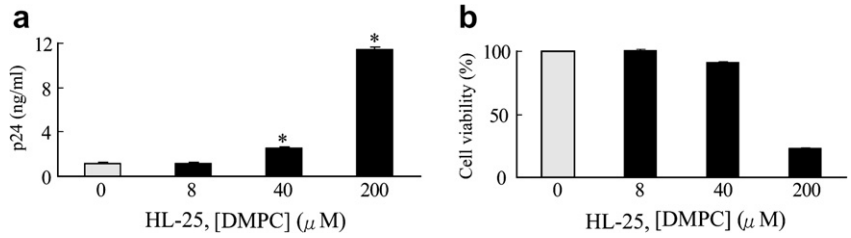


Figure 3. Effects of HL-25 on HIV-1 production in J_{22}-HL-60 cells. (a) Virus production of J_{22}-HL-60 cells treated with HL-25. *Significant difference ($p < 0.05$) compared with control (Student's *t*-test). (b) Cell viability of J_{22}-HL-60 cells after the treatment with HL-25. The concentration ranges were [DMPC] = 8–200 μM and [$C_{12}(EO)_{25}$] = 0.9–22 μM for DMPC and $C_{12}(EO)_{25}$, respectively. Data represent the mean ± SD.

fected cells efficiently. Thus, we next addressed whether the HL-25 induces the virus production in latently infected cells on the basis of the HIV-1 p24 chemiluminescent enzyme immunoassay (CLEIA; Lumipulse, Fujirebio Inc., Japan) according to the manufacturer's protocol.[27] The human J_{22}-HL-60 latently HIV-1 (JRFL)-infected cells were derived from promyelocytic leukemia (HL-60) cells, and constantly produce low levels of supernatant p24.[28] J_{22}-HL-60 cells were cultured for 72 h in the presence of HL-25.[14] The production of p24 in J_{22}-HL-60 cells was increased in the presence of HL-25 as the DMPC concentration was raised, as shown in Figure 3a. Especially, the markedly high production of p24 (11.2 ng/ml) was attained at 200 μM of DMPC concentration, which was 9.0 times more than that of the control. The proliferation of the cells was also inhibited by HL-25 at 200 μM of DMPC concentration (Fig. 3b). These observations indicate that HL-25 should have the remarkable high production effects of HIV-1 secreted by J_{22}-HL-60 cells in association with cell growth retardation. We have already reported that HL inhibits the growth of uninfected HL-60 cells leading to apoptosis.[8] Therefore, these results suggest that HL-25 having the activity of HIV-1 production should induce apoptosis for latently virus infected cells. This further indicates that HL-25 could be used as a much safer adjuvant as compared to aforementioned factors.

In conclusion, we clearly demonstrated the marked inhibitory effects of DMPC liposomes and HL on the growth of HIV-1 chronically infected (MOLT-4/IIIB) cells in vitro. It is worthy to note that a good correlation between the membrane fluidity of HL and their inhibitory effects on the growth of MOLT-4/IIIB cells was observed for the first time. Furthermore, we clarified that HL-25 should have remarkable inducing ability for HIV-1 from latently infected (J_{22}-HL-60) cells leading to growth inhibition. Thus, this study emphasized that growth inhibition and virus production for HIV-1 chronically infected cells by HL might provide the possibility of novel therapeutic approaches for the elimination of these infected cells.

Acknowledgment

This work was supported in part by a Grant-in-Aid for Science Research from the Ministry of Education, Science, and Culture of Japan (No. 17360403, 18031041).

References and notes

1. Folks, T. M.; Clouse, K. A.; Justement, J.; Rabson, A.; Duh, E.; Kehrl, J. H.; Fauci, A. S. *Proc. Natl. Acad. Sci. USA* **1989**, *86*, 2365.
2. Pomerantz, R. J.; Feinberg, M. B.; Trono, D.; Baltimore, D. *J. Exp. Med.* **1990**, *174*, 253.
3. Finzi, D.; Hermankova, M.; Pierson, T.; Carruth, L. M.; Buck, C.; Chaisson, R. E.; Quinn, T. C.; Chadwick, K.; Margolick, J.; Brookmeyer, R.; Gallant, J.; Markowitz, J.; Ho, D. D.; Richman, D. D.; Siliciano, R. F. *Science* **1997**, *278*, 1295.
4. Crowe, S. M.; Sonza, S. *J. Leuk. Biol.* **2000**, *68*, 345.
5. Ueoka, R.; Matsumoto, Y.; Moss, R. A.; Swarup, S.; Sugii, A.; Harada, K.; Kikuchi, J.; Murakami, Y. *J. Am. Chem. Soc.* **1988**, *110*, 1588.
6. Matsumoto, Y.; Kato, T.; Kemura, Y.; Tsuchiya, M.; Yamamoto, M.; Ueoka, R. *Chem. Lett.* **1999**, 53.
7. Ueoka, R.; Matsumoto, Y.; Ichihara, H.; Kiyokawa, T. *Am. Chem. Soc. Books*; Marten, M. R.; Park T. H.; Nagamune, T., Eds.; Am. Chem. Soc. Symposium Series 830: Washington, 2002, pp. 177–189.
8. Matsumoto, Y.; Iwamoto, Y.; Ueoka, R. *Int. J. Cancer* **2005**, *115*, 377.
9. Ichihara, H.; Nagami, H.; Kiyokawa, T.; Matsumoto, Y.; Ueoka, R. *Anticancer Res.* **2008**, *28*, 1187.
10. Nakano, K.; Iwamoto, Y.; Takata, W.; Matsumoto, Y.; Ueoka, R. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3251.
11. Komizu, Y.; Matsumoto, Y.; Ueoka, R. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 6131.
12. Huang, S. K.; Lee, K. D.; Hong, K.; Friend, D. S.; Papahadjopoulos, D. *Cancer Res.* **1992**, *52*, 5135.
13. Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55.
14. MOLT-4, MOLT-4/IIIB [MOLT-4/HIV-1 human T cell leukemia virus (HTLV)-IIIB][15] and J_{22}-HL-60 cells[28] were maintained in RPMI-1640 medium supplemented with 10% FCS (fetal calf serum) and antibiotics (100 μg/ml penicillin/100 μg/ml streptomycin).
15. Kikukawa, R.; Koyanagi, Y.; Harada, S.; Kobayashi, N.; Hatanaka, M.; Yamamoto, N. *J. Virol.* **1986**, *57*, 1159.
16. Aloia, R. C.; Tian, U.; Jensen, F. C. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 5181.
17. Inbar, M. *FEBS Lett.* **1976**, *67*, 180.
18. Shinitzky, M. *Biochim. Biophys. Acta* **1984**, *738*, 251.
19. The emission at 430 nm originating from DPH was monitored upon excitation at 360 nm using a fluorescence polarization spectrophotometer (Hitachi F-2000). *P* of DPH into HL was calculated by $P = (I_{vv} - C_f I_{vh})/(I_{vv} + C_f I_{vh})$ Where *I* is the fluorescence intensity and the subscripts v and h refer to the orientations, vertical and horizontal, respectively, for the excitation and analyzer polarizers in this sequence: for example, I_{vh} indicates the fluorescence intensity measured with a vertical excitation polarizer and a horizontal analyzer polarizer.[20] C_f is the grating correction factor, given by I_{hv}/I_{hh}.
20. Azumi, T.; McGlynn, S. P. *J. Chem. Phys.* **1962**, *37*, 2413.
21. Scheller, C.; Sopper, S.; Chen, P.; Flory, E.; Koutsilieri, E.; Racek, T.; Ludwig, S.; ter Meulen, V.; Jassoy, C. *J. Biol. Chem.* **2002**, *277*, 15459.
22. Takahashi, Y.; Tanaka, Y.; Yamashita, A.; Koyanagi, Y.; Nakamura, M.; Yamamoto, N. *J. Virol.* **2001**, *75*, 6748.
23. Harada, S.; Yamamoto, N.; Fujiki, H. *AIDS Res. Hum. Retroviruses* **1988**, *4*, 99.
24. Hamer, D. H.; Bocklandt, S.; McHugh, L.; Chun, T.-W.; Blumberg, P. M.; Sigano, D. M.; Marquez, V. E. *J. Virol.* **2003**, *77*, 10227.
25. Papp, B.; Byrn, R. A. *J. Biol. Chem.* **1995**, *270*, 10278.
26. Rivas, C. I.; Vera, J. C.; Guaiquil, V. H.; Velasquez, F. V.; Borquez-Ojeda, O. A.; Carcamo, J. G.; Concha, I. I.; Golde, D. W. *J. Biol. Chem.* **1997**, *272*, 5814.
27. Sakai, A.; Hirabayasi, Y.; Aizawa, S.; Tanaka, M.; Ida, S.; Oka, S. *J. Jap. Assoc. Infect. Dis.* **1999**, *73*, 205.
28. Kitano, K.; Baldwin, G. C.; Raines, M. A.; Golde, D. W. *Blood* **1990**, *76*, 1980.