Biochemical and Biophysical Research Communications 457 (2015) 288–294

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Inhibition of HIV-1 entry by the tricyclic coumarin GUT-70 through the modification of membrane fluidity

Kouki Matsuda [a], Shinichiro Hattori [a], Ryusho Kariya [a], Yuji Komizu [b], Eriko Kudo [a], Hiroki Goto [a], Manabu Taura [a], Ryuichi Ueoka [b], Shinya Kimura [c], Seiji Okada [a, *]

[a] Division of Hematopoiesis, Center for AIDS Research, Kumamoto University, 2-2-1 Honjo, Chuo-ku, Kumamoto 860-0811, Japan
[b] Division of Applied Life Science, Graduate School of Engineering, Sojo University, 4-22-1 Ikeda, Nishi-ku, Kumamoto 860-0082, Japan
[c] Division of Hematology, Respiratory Medicine and Oncology, Department of Internal Medicine, Faculty of Medicine, Saga University, 5-1-1 Nabeshima, Saga 849-8501, Japan

ARTICLE INFO

Article history:
Received 8 December 2014
Available online 7 January 2015

Keywords:
HIV-1 infection
Viral entry
Cell fusion
Membrane fluidity
GUT-70

ABSTRACT

Membrane fusion between host cells and HIV-1 is the initial step in HIV-1 infection, and plasma membrane fluidity strongly influences infectivity. In the present study, we demonstrated that GUT-70, a natural product derived from *Calophyllum brasiliense*, stabilized plasma membrane fluidity, inhibited HIV-1 entry, and down-regulated the expression of CD4, CCR5, and CXCR4. Since GUT-70 also had an inhibitory effect on viral replication through the inhibition of NF-κB, it is expected to be used as a dual functional and viral mutation resistant reagent. Thus, these unique properties of GUT-70 enable the development of novel therapeutic agents against HIV-1 infection.

1. Introduction

Membrane fusion between host T cells and human immunodeficiency virus type 1 (HIV-1) is one of the essential steps in HIV-1 infection. HIV-1 enters cells by binding of the HIV-1 envelope protein, gp120 and CD4 on CD4+ T cells or macrophages as a trigger of infection, and gp120 then interacts with the chemokine receptors CXCR4 and CCR5, leading to infection by HIV-1 [1]. HIV-1 destroys CD4+ T cells post-infection through a stage of a relatively long latent period [2–4]. Combination antiretroviral therapy (cART), which combines two reverse transcriptase inhibitors and protease inhibitors or integrase inhibitors, targets the virus life cycle and has successfully reduced the morbidity and mortality caused by HIV-1 infection [5,6]. Although HIV-1 infection can be controlled for prolonged periods, there is currently no fundamental treatment to eradicate the virus from the body. Moreover, the emergence of drug-resistant viruses is also one of the main difficulties associated with the treatment of HIV-1 infection. Novel therapeutic approaches that are distinct from those of currently available anti-retroviral drugs are essential for the continual control of HIV-1 infection.

The genus *Calophyllum* comprises ca. 180 tree species with a pantropical distribution, and dipyrano-tetracyclic coumarins that are active against HIV-1 have been isolated from several *Calophyllum* species. Previous studies on *Calophyllum* species including *Calophyllum brasiliense* demonstrated their inhibiting effects on HIV-1 reverse transcriptase and viral replication [7–9]. The newly discovered anti-tumor agent GUT-70, a natural product derived from the stem bark of *C. brasiliense*, has been characterized as a tricyclic coumarin with the formula 5-methoxy-2, 2-dimethyl-6-(2-methyl-1-oxo-2-butenyl)-10-propyl-2*H*,8*H*-benzo[1,2-*b*;3,4-*b*0] dipyran-8-one($C_{23}H_{26}O_5$). GUT-70 exhibits cytotoxic effects against human leukemia [10] and lymphoma [11]. We also reported that GUT-70 inhibited HIV-1 replication in chronically infected cells via suppression of the NF-κB pathway [12]. Thus, the multiple functions of GUT-70 may be applied to the treatment of both infectious diseases and malignancies.

Viral entry is known to be influenced by cell membrane fluidity [13] and previous studies reported that glycyrrhizin and cepharanthine, derived from natural products, affected HIV-1 infectivity by modifying membrane fluidity [13–15]. Since GUT-70 decreased cell membrane fluidity, we herein focused on the effects of GUT-70 against the HIV-1 entry process. The results obtained clearly demonstrated that GUT-70 inhibited HIV-1 viral entry by reducing cell membrane fluidity.

Abbreviations: HIV-1, human immunodeficiency virus-1; cART, combination antiretroviral therapy; DPH, 1,6-diphenyl-1,3,5-hexatriene.

* Corresponding author. Fax:+81 96 373 6523
E-mail address: okadas@kumamoto-u.ac.jp (S. Okada).

http://dx.doi.org/10.1016/j.bbrc.2014.12.102
0006-291X/

2. Materials and methods

2.1. Preparation of GUT-70

GUT-70 used in the present study was synthesized by Nippon Shinyaku Co. (Kyoto, Japan), its structure was confirmed with NMR and an elemental analyzer, and its purity was confirmed with HPLC (purity 97.72%) [10,12].

2.2. Cell lines and culture

The human T cell lines, MOLT-4, $Jurkat_{HXBc2\ (4)}$, and $Jurkat_{522F/Y}$ were obtained through the NIH AIDS Research and Reference Reagent Program (Germantown, MD). PM1-CCR5 cells stably expressing human CCR5 were a gift from Dr. Y. Maeda (Kumamoto Univ., Kumamoto, Japan) [16]. MOLT-4 and PM1-CCR5 cells were maintained in RPMI-1640 medium (Gibco, Gaithersburg, MD) supplemented with penicillin 100 U/ml, streptomycin 100 μg/ml, and 10% fetal bovine serum (HyClone Laboratories, Logan, UT). These cells were cultured in a 5% CO_2 humidified incubator at 37 °C. $Jurkat_{HXBc2\ (4)}$ and $Jurkat_{522F/Y}$ were stably transfected with the Env gene from the $HIV\text{-}1_{HXBc2}$ strain. $Jurkat_{HXBc2\ (4)}$ expresses a functional gp120/gp41 glycoprotein(*Env*), while $Jurkat_{522F/Y}$ contains an F/Y substitution at position 522 in gp41 that prevents fusion [17]. These cell lines were cultured in RPMI-1640 supplemented with 10% FBS (Gibco BRL), 100 U/ml penicillin, 100 μg/ml streptomycin, 1 μg/ml tetracycline, 200 μg/ml G418, and 200 μg/ml hygromycin. The expression of Env was induced by the removal of tetracycline by washing cells with PBS, and culturing for 3 days in medium without tetracycline before the fusion experiments.

2.3. Measurement of cell membrane fluidity

Cell membrane fluidity was measured using a spectrophotofluorometer (F4500, HITACHI, Japan) on the basis of the fluorescent depolarization method [18,19]. Briefly, MOLT-4 and PM1-CCR5 T cell lines (2.5×10^6 cells) were labeled with DPH (1,6-diphenyl-1,3,5-hexatriene, Wako, Japan) at a final concentration of 2×10^{-6} M and were incubated at 37 °C under light-shielded conditions for 30 min. After labeling with DPH, cells were washed with PBS and 2.5×10^5 cells/ml was prepared using PBS. GUT-70 was added to cells at final concentrations of 0, 10, 50, and 100 μM. The fluorescence polarization degree (P value) was measured for 5 min following the addition of GUT-70.

2.4. Cell–cell fusion inhibition assay

Env-expressing cell lines ($Jurkat_{HXBc2\ (4)}$ and $Jurkat_{522F/Y}$) and the MOLT-4 T cell line were labeled with the PKH67 Green Fluorescent Cell Linker Kit (Sigma, St. Louis, MO) and PKH26 Red Fluorescent Cell Linker Kit (Sigma), respectively. MOLT4 cells were treated with GUT-70 at final concentrations of 0–10 μM for 1 h. The two cell populations were then co-cultured at a ratio of 1:1 for 24 h. Cells were analyzed using an LSR II flow cytometer (BD Bioscience, San Jose, CA). PKH67 and PKH26 double positive cells were defined as fused cells. Data were analyzed with FlowJo (Tree Star, San Carlos, CA) software. Cells were also observed using fluorescent microscopy (Biozero, KEYENCE, Japan) after 48 h.

2.5. Virus infection and flow cytometric detection of HIV-1 infected cells

293T cells were maintained in DMEM containing 10% FCS. The virus was produced from 293T cells by transfection with the proviral plasmid $HIV\text{-}1_{NL4\text{-}3}$ (NIH AIDS Research and Reference Reagent Program) or JR-FL (kindly provided by Prof. Y. Koyanagi, Kyoto Univ., Kyoto Japan) using Hily Max (Dojin Chemical, Kumamoto, Japan). The culture supernatant was collected, the p24 value was measured using the p24 antigen ELISA kit (ZeptoMetrix Corp., Buffalo, NY), and it was then stored at −80 °C until use.

2.6. Virus infection and flow cytometric detection of HIV-1 infected cells

The PM1-CCR5 T cell line (5×10^5 cells/ml) was treated with GUT-70 at final concentrations of 0–100 μM and was then incubated for 10 min at 37 °C. Cells were infected with $HIV\text{-}1_{NL4\text{-}3}$ (X4 tropic) (p24 concentration; 25 ng/ml) or $HIV\text{-}1_{JR\text{-}FL}$ (R5 tropic) (p24 concentration; 50 ng/ml) for 1 h, washed twice with PBS, and then cultured for 48 h. HIV-1 infected cells (intracellular p24 positive) were detected by flow cytometry [20]. Briefly, cells were stained with anti-human CD4-APC mAb (clone: RPA-T4) (BioLegend, San Diego, CA). After 30 min of being incubated on ice, cells were washed twice with washing medium, fixed with 1% paraformaldehyde/PBS for 20 min in the dark, and permeabilized with 0.1% saponin/PBS. After 10 min of being incubated on ice, cells were stained with anti-HIV-1 Gag p24-FITC mAb (Beckman Coulter, Fullerton, CA) for 30 min on ice. Cells were then analyzed on an LSR II flow cytometer. Data were analyzed with FlowJo software.

2.7. Quantification of HIV-1 p24 in cell culture supernatants

The amount of the p24 antigen in cell culture supernatants was determined using an HIV-1 p24 antigen ELISA kit [20].

2.8. Quantitative viral DNA analysis

Total DNA was extracted to detect HIV-1 proviral DNA by quantitative-PCR [21,22]. Quantitative PCR analyses for HIV-1 Gag and internal controls human GAPDH were performed using the SYBR Green Master Mix (Applied Biosystems, Carlsbad, CA) and StepOne real-time PCR system (Applied Biosystems). Primer sequences were as follows: HIV Gag: 5′-AGTGGGGGGA-CATCAAGCAGCCATGCAAAT, 5′-TACTAGTAGTTCCTGCTATGT-CACTTCC; hGAPDH: 5′-CGGGAAGCTTGTGATCAATGG, 5′-GGCAGTGATGGCATGGACTG.

2.9. Entry assay

The entry process of virions into cells was quantitatively analyzed by Gag p24 ELISA, as previously described [23,24]. Briefly, PM-1 CCR5 T cell lines (5×10^5 cells/ml) were treated with GUT-70 for 10 min, infected with $HIV\text{-}1_{NL\text{-}43}$ and $HIV\text{-}1_{JR\text{-}FL}$ for 1 h at 37 °C, trypsinized for 5 min, washed extensively, lysed with NP-40, and the internalized virion HIV-1 Gag p24 protein was then measured using a HIV-1 p24 antigen ELISA kit (ZeptoMetrix Corp., Buffalo, NY).

2.10. Flow cytometric analysis of receptor expression

PM1-CCR5 T cell lines (5×10^5 cells/ml) were treated with GUT-70 at final concentrations of 0, 50, and 100 μM and were incubated for 1 h at 37 °C. Cells were washed twice with washing medium for extracellular or intracellular staining. The extracellular antigens were stained with anti-human CD4-Pacific Blue mAb (clone: RPA-T4), anti-human CXCR4-APC mAb (clone: 12G5) (BioLegend), and anti-human CCR5-PE mAb (clone: HEK/1/85a) (BioLegend) for 30 min on ice. Cells were fixed with 1% paraformaldehyde/PBS for 20 min in the dark, and permeabilized with 0.1% saponin/PBS. After

10 min of being incubated on ice, intracellular antigens were stained with the above mAbs for 30 min on ice. Cells were analyzed on an LSR II flow cytometer. Data were analyzed with FlowJo software.

2.11. RT-PCR analysis

Total RNA was extracted from cells using RNAiso Plus (Takara, Japan). First-strand cDNA was synthesized from RNA with Oligo-dT prier and AMV Reverse Transcriptase XL and then amplified with Ex-Taq (Takara Bio, Otsu, Japan). The PCR products were analyzed with 2.0% agarose gel electrophoresis and ethidium bromide staining. Primer sequences were as follows: CD4: 5′-GCAGTGGC-GAGCTGTGGT [25], 5′-GAGGAGGCGAACAGGA-GG; CCR5: 5′-CGTCTCTCCCAGGAATCATCTTTAC, 5′-TTGAGTCCGTGT-CACAAGCCC [26]; CXCR4: 5′-TGACTCCATGAAGGAACCCTG, 5′-CTTGGCC-TCTGACTGTTGGTG [27]; G3PDH: 5′-TGAAGGTCGGAGTCAACG-GATTTGG-T, 5′-CATGTGGGCCATGAGGTCCACCAC.

2.12. Statistical analysis

Parametric statistical analyses were performed using the Student's *t*-test. *P* values less than 0.05 were defined as significant.

3. Results

3.1. GUT-70 decreased cell membrane fluidity

Viral entry is influenced by cell membrane fluidity [13]; therefore, we assessed the effects of GUT-70 on cell membrane fluidity using the fluorescent depolarization method [18,19]. Fluorescence depolarization (P) measurements of DPH as a fluorescence probe can be used to examine lipid interactions or the fusion of other membrane systems with different lipid fluidities. When treated with GUT-70, the cell membrane fluidities of the MOLT-4 and PM1-CCR5 T cell lines decreased in a dose-dependent manner (Fig. 1).

3.2. GUT-70 inhibited HIV-1 envelope-dependent cell fusion

The fusion of cell membranes between a virus-infected cell and target cell is an essential step in HIV-1 infection. Therefore, we evaluated the effects of GUT-70 on HIV-1 envelope-dependent cell fusion using a fusion model system [15,17]. As shown in Fig. 3A, PKH67 green fluorescence and PKH26 red fluorescence double positive cells (fused cells) were decreased in a dose-dependent manner with the GUT-70 treatment (Fig. 2A). GUT-70 also significantly inhibited cell fusion ($p < 0.001$) (Fig. 2B). We subsequently

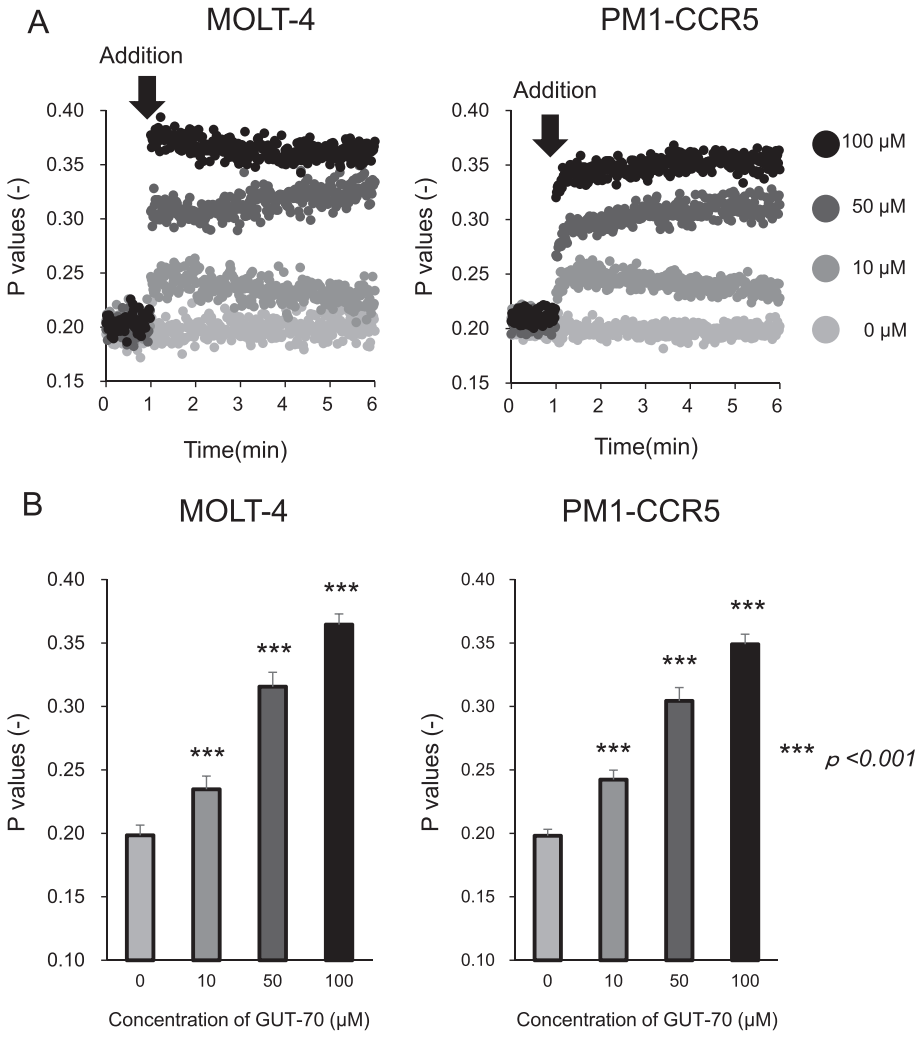

Fig. 1. Membrane fluidity of MOLT-4 cells and PM1-CCR5 cells treated with GUT-70. (A) The fluorescence polarization of DPH-labeled MOLT-4 cells and PM1-CCR5 cells treated with GUT-70 (0–100 μM) was continuously measured using a fluorescence spectrophotometer. Results are representative of three independent experiments. (B) Mean P values during 4–5 min.

observed fused cells with a fluorescent microscope. The number of yellow colored cells (fused cells) was decreased by the GUT-70 treatment (Fig. 2C). These results indicated that GUT-70 inhibited HIV-1 envelope dependent cell–cell fusion and suggested that GUT-70 inhibited cell-virus fusion.

3.3. GUT-70 inhibited HIV-1 infection

We infected the PM1-CCR5 T cell line with HIV-1 in the presence or absence of GUT-70. PM1-CCR5 cells are a human CD4-and human CXCR4/CCR5-positive T cell line that can be infected by X4 tropic and R5 tropic HIV-1 strains [16]. PM1-CCR5 cells were exposed to X4 tropic NL4-3 HIV-1 with or without GUT-70. Cells and supernatants were harvested at 48 h post-infection. The intracellular expression of p24 and HIV-1 production as measured with p24 ELISA was decreased by the GUT-70 treatment in a dose-dependent manner (Fig. 3A–C). Furthermore, we confirmed the amount of viral DNA that was integrated into the host cell genome using quantitative-PCR. The amount of integrated viral DNA was also reduced by the treatment with GUT-70 (Fig. 3D). We next performed a viral entry assay to confirm that GUT-70 inhibited the entry step of HIV-1 [23,24]. As shown in Fig. 3E, viral entry was significantly inhibited in NL4-3 HIV-1 strain by the treatment with GUT-70 in a dose-dependent manner. When PM1-CCR5 cells were exposed to R5 tropic JRFL HIV-1, GUT-70 also inhibited HIV-1 viral entry (Supplemental Fig. 1). These results indicated that GUT-70 inhibited HIV-1 infection by X4 tropic and R5 tropic viruses through the inhibition of viral entry.

3.4. GUT-70 down-regulated the expression of CD4, CXCR4, and CCR5

We measured the expression levels of the HIV-1 receptor (CD4) and co-receptors (CXCR4 and CCR5) on the cell surface in order to elucidate the mechanism underlying the inhibition of entry by HIV-1. The expression levels of CD4, CXCR4, and CCR5 on the cell surface were significantly down-regulated by the GUT-70 treatment in a dose-dependent manner (Fig. 4A and B). Semi-quantitative RT-PCR

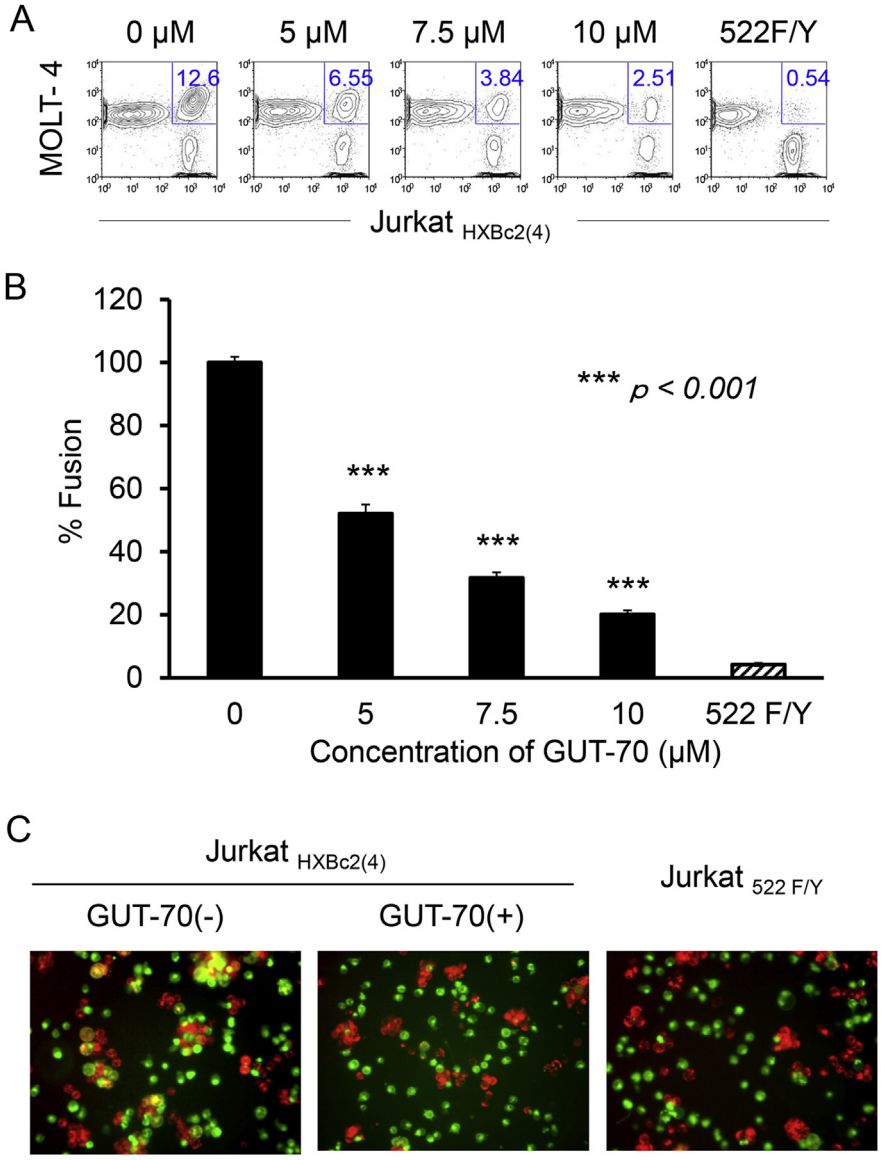

Fig. 2. Inhibition of Env-dependent cell-to-cell fusion by GUT-70. (A) The inhibition of cell fusion was analyzed by flow cytometry. (B) Summary of (A). (C) Images of cell fusion under fluorescent microscopy (×800). Data represents the mean ± SD. One representative result from 3 independent experiments is shown.

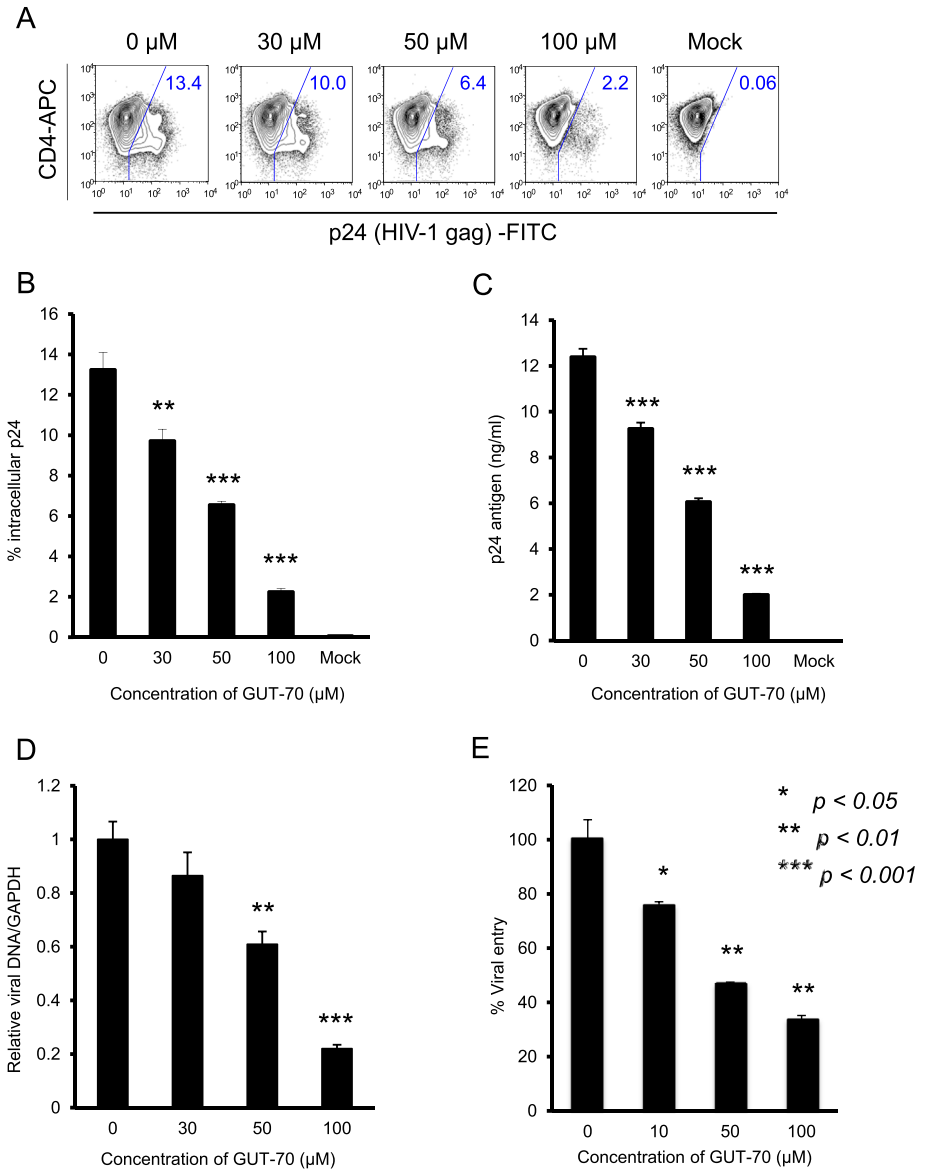

Fig. 3. Inhibition of HIV-1 infection by GUT-70 at the viral entry step. (A, B) HIV-1_{NL-43}-infected PM1-CCR5 cells were analyzed with flow cytometry at 48 h post-infection. The intracellular p24 (HIV-1 Gag) value of GUT-70-treated PM1-CCR5 cells. (C) Viral production by GUT-70-treated PM1-CCR5 cells was detected by ELISA. (D) The quantity of integrated HIV DNA was determined by Quantitative-PCR analysis relative to the positive control (non-treated cells). (E) The PM-1 CCR5 T cell line (5×10^5 cells/ml) was treated with GUT-70 for 10 min and infected with HIV-1_{NL-43} for 1 h. Cells were trypsinized, lysed with NP-40, and the internalized virion HIV-1 Gag p24 protein was measured with ELISA. Data represents the mean ± SD. One representative result from 3 independent experiments is shown.

revealed that the mRNA levels of CD4, CXCR4, and CCR5 did not change with the GUT-70 treatment (Fig. 4C). These results indicated that the down-regulated expression of the cell surface HIV-1 receptor and co-receptors modified HIV-1 entry.

4. Discussion

The plasma membrane is the first barrier to HIV-1 infection and membrane fluidity is known to strongly influence the viral entry step of HIV-1 [13,28]. During screening processes, we found that GUT-70 stabilized membrane fluidity. Further analyses revealed that GUT-70 inhibited the HIV-1 entry step in part by down-regulating the expression of the HIV-1 receptor and co-receptors. As productive HIV-1 infection depends on the initial interaction of the virus with the host plasma membrane, modifications to the host plasma membrane such as that by the GUT-70 treatment may lead to the development of novel therapeutic approaches.

Fusion between the HIV-1 envelope and host cell plasma membrane is a key step in viral entry [29] and the modulation of plasma membrane fluidity alters the lipid raft, plasma membrane lipid microdomains [28]. Since the HIV entry receptors are preferentially localized in lipid rafts and lipid rafts drive gp120-gp41, CD4, and chemokine receptors into a membrane fusion process [30], the modulation of lipid rafts may contribute to the inhibition of viral entry. We demonstrated that the GUT-70 treatment suppressed the surface expression of CD4, CXCR4, and CCR5 (Fig. 4). However, these levels of suppression may not be sufficient to explain the phenomenon. Other mechanisms to inhibit HIV-1 entry may exist through the modulation of plasma membrane fluidity and lipid rafts. The elucidation of this mechanism in more detail will lead to the development of novel membrane targeting therapies for HIV-1 and other viral infections.

Coumarins are plant-derived natural products that have multiple pharmacological properties including anti-viral effects [31].

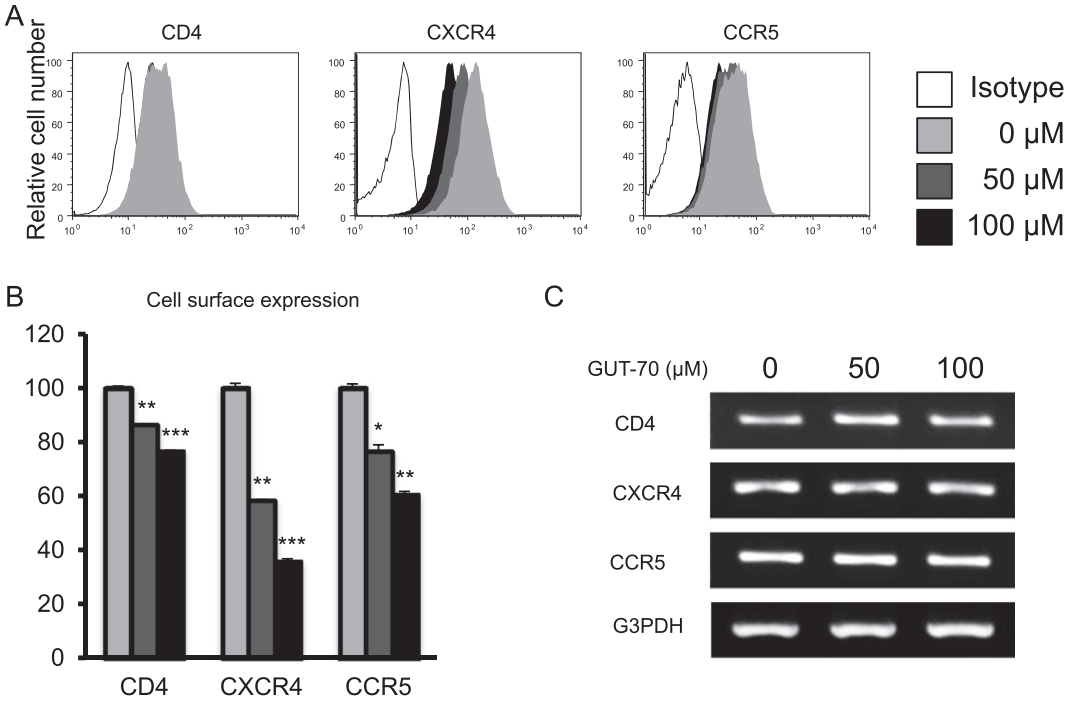

Fig. 4. Down-regulation of CD4, CXCR4, and CCR5 with GUT-70. PM1-CCR5 cells were treated with GUT-70 for 1 h. Cells were stained with mAbs against CD4, CXCR4, and CCR5. (A) Expression levels were analyzed by flow cytometry. (B) Bar graph represent the mean fluorescence intensity of each surface molecule. Data represents the mean ± SD. (C) The mRNA levels of CD4, CXCR4, and CCR5 were analyzed by semi-quantitative RT-PCR.

Previous studies reported that coumarin compounds inhibited viral reverse transcriptase and viral replication in HIV-1 infection [7–9]. We also showed that GUT-70 inhibited viral replication in acutely and chronically infected cells via the suppression of NF-κB [12]. In the present study, we demonstrated that GUT-70 strongly suppressed the viral entry step. Thus, GUT-70 has dual effects on HIV-1 infection, indicating that GUT-70 has the unique property of being an anti-HIV-1 reagent with the weak potential to induce drug-resistant clones. Furthermore, since the anti-viral mechanisms of GUT-70 are distinct from those of currently available anti-retroviral drugs, it can be used in combination therapy with these reagents.

The introduction of combined antiretroviral therapy has reduced mortality in HIV-1-infected patients [32]. However, malignant lymphoma is still a severe and frequent complication in HIV-1-infected patients [33,34]. AIDS-related lymphoma has an extremely aggressive clinical course [35,36] and most anti-tumor reagents have drug–drug interactions with anti-HIV-1 reagents [37], leading to difficulties in the treatment of AIDS-related lymphoma. Since GUT-70 has anti-lymphoma and leukemia activities [10,11] in addition to its anti-HIV-1 effects, it is expected to be applied as a key drug in the prevention and treatment of related malignancies.

5. Conclusion

We confirmed that GUT-70 inhibited the HIV-1 entry process by reducing cell membrane fluidity. The down-regulated expression of the HIV-1 receptor CD4 and co-receptors CXCR4 and CCR5 may contribute to this inhibition. Since GUT-70 is known to have anti-HIV-1 replication and anti-lymphoma effects, it can be used as a dual effect anti-HIV-1 reagent in the treatment of AIDS-related lymphoma. The results of the present study suggest that host cell membrane-targeting therapies have potentially beneficial effects against HIV-1 early infection, and provide a platform for novel therapeutic approaches to prevent HIV-1 infection.

Conflict of interest

The authors declare no competing financial interests.

Contributions

K.M. and S.O. designed the research; K.M., S.H., R.K., Y.K., E.K., H.G., and M.T. performed the research; R.U., and S.K. contributed reagents/materials/analysis tools and interpreted the data; K.M. and S.O. wrote the manuscript.

Acknowledgments

We thank Ms. I. Suzu and Ms. S. Fujikawa for their technical assistance and Ms. Y. Endo for her secretarial assistance. This work was supported in part by a Health and Labour Sciences Research Grant from the Ministry of Health, Labour, and Welfare of Japan (H25-AIDS-I-002), by the Global COE program, “Global Education and Research Center Aiming at the Control of AIDS”, Grants-in-Aid for Science Research (No. 25114711) from the Ministry of Education, Science, Sports, and Culture of Japan, and a scholarship for the Graduate School of Medical Sciences, Kumamoto University, Japan.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2014.12.102.

References

[1] M. Stevenson, HIV-1 pathogenesis, Nat. Med. 9 (2003) 853–860.
[2] E.A. Berger, P.M. Murphy, J.M. Farber, Chemokine receptors as HIV-1 coreceptors: roles in viral entry, tropism, and disease, Annu. Rev. Immunol. 17 (1999) 657–700.

[3] R.I. Connor, K.E. Sheridan, D. Ceradini, S. Choe, N.R. Landau, Change in coreceptor use correlates with disease progression in HIV-1–infected individuals, J. Exp. Med. 185 (1997) 621–628.
[4] D.R. Littman, Chemokine receptors: keys to AIDS pathogenesis? Cell 93 (1998) 677–680.
[5] R.M. Gulick, J.W. Mellors, D. Havlir, J.J. Eron, C. Gonzalez, D. McMahon, D.D. Richman, F.T. Valentine, L. Jonas, A. Meibohm, E.A. Emini, J.A. Chodakewitz, Treatment with indinavir, zidovudine, and lamivudine in adults with human immunodeficiency virus infection and prior antiretroviral therapy, N. Engl. J. Med. 337 (1997) 734–739.
[6] F.J. Palella Jr., K.M. Delaney, A.C. Moorman, M.O. Loveless, J. Fuhrer, G.A. Satten, D.J. Aschman, S.D. Holmberg, Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators, N. Engl. J. Med. 338 (1998) 853–860.
[7] Y. Kashman, K.R. Gustafson, R.W. Fuller, J.H. Cardellina 2nd, J.B. McMahon, M.J. Currens, R.W. Buckheit Jr., S.H. Hughes, G.M. Cragg, M.R. Boyd, The calanolides, a novel HIV-inhibitory class of coumarin derivatives from the tropical rainforest tree, *Calophyllum lanigerum*, J. Med. Chem. 35 (1992) 2735–2743.
[8] M. Huerta-Reyes, C. Basualdo Mdel, F. Abe, M. Jimenez-Estrada, C. Soler, R. Reyes-Chilpa, HIV-1 inhibitory compounds from *Calophyllum brasiliense* leaves, Biol. Pharm. Bull. 27 (2004) 1471–1475.
[9] G.Z. Cesar, M.G. Alfonso, M.M. Marius, E.M. Elizabeth, C.B. Angel, H.R. Maira, C.L. Guadalupe, J.E. Manuel, R.C. Ricardo, Inhibition of HIV-1 reverse transcriptase, toxicological and chemical profile of *Calophyllum brasiliense* extracts from Chiapas, Mexico, Fitoterapia 82 (2011) 1027–1034.
[10] S. Kimura, C. Ito, N. Jyoko, H. Segawa, J. Kuroda, M. Okada, S. Adachi, T. Nakahata, T. Yuasa, V.C. Filho, H. Furukawa, T. Maekawa, Inhibition of leukemic cell growth by a novel anti-cancer drug (GUT-70) from *Calophyllum brasiliense* that acts by induction of apoptosis, Int. J. Cancer 113 (2005) 158–165.
[11] L. Jin, Y. Tabe, S. Kimura, Y. Zhou, J. Kuroda, H. Asou, T. Inaba, M. Konopleva, M. Andreeff, T. Miida, Antiproliferative and proapoptotic activity of GUT-70 mediated through potent inhibition of Hsp90 in mantle cell lymphoma, Br. J. Cancer 104 (2011) 91–100.
[12] E. Kudo, M. Taura, K. Matsuda, M. Shimamoto, R. Kariya, H. Goto, S. Hattori, S. Kimura, S. Okada, Inhibition of HIV-1 replication by a tricyclic coumarin GUT-70 in acutely and chronically infected cells, Bioorg. Med. Chem. Lett. 23 (2013) 606–609.
[13] S. Harada, K. Yusa, K. Monde, T. Akaike, Y. Maeda, Influence of membrane fluidity on human immunodeficiency virus type 1 entry, Biochem. Biophys. Res. Commun. 329 (2005) 480–486.
[14] S. Harada, The broad anti-viral agent glycyrrhizin directly modulates the fluidity of plasma membrane and HIV-1 envelope, Biochem. J. 392 (2005) 191–199.
[15] K. Matsuda, S. Hattori, Y. Komizu, R. Kariya, R. Ueoka, S. Okada, Cepharanthine inhibited HIV-1 cell-cell transmission and cell-free infection via modification of cell membrane fluidity, Bioorg. Med. Chem. Lett. 24 (2014) 2115–2117.
[16] K. Yusa, Y. Maeda, A. Fujioka, K. Monde, S. Harada, Isolation of TAK-779-resistant HIV-1 from an R5 HIV-1 GP120 V3 loop library, J. Biol. Chem. 280 (2005) 30083–30090.
[17] J. Cao, I.W. Park, A. Cooper, J. Sodroski, Molecular determinants of acute single-cell lysis by human immunodeficiency virus type 1, J. Virol. 70 (1996) 1340–1354.
[18] T. Towata, Y. Komizu, S. Suzu, R. Ueoka, S. Okada, Highly selective fusion and accumulation of hybrid liposomes into primary effusion lymphoma cells along with induction of apoptosis, Biochem. Biophys. Res. Commun. 393 (2010) 445–448.
[19] A. Levanon, A. Kohn, M. Inbar, Increase in lipid fluidity of cellular membranes induced by adsorption of RNA and DNA virions, J. Virol. 22 (1977) 353–360.
[20] S. Hattori, K. Ide, H. Nakata, H. Harada, S. Suzu, N. Ashida, S. Kohgo, H. Hayakawa, H. Mitsuya, S. Okada, Potent activity of a nucleoside reverse transcriptase inhibitor, 4'-ethynyl-2-fluoro-2'-deoxyadenosine, against human immunodeficiency virus type 1 infection in a model using human peripheral blood mononuclear cell-transplanted NOD/SCID Janus kinase 3 knockout mice, Antimicrob. Agents Chemother. 53 (2009) 3887–3893.
[21] Y. Li, L. Li, R. Wadley, S.W. Reddel, J.C. Qi, C. Archis, A. Collins, E. Clark, M. Cooley, S. Kouts, H.M. Naif, M. Alali, A. Cunningham, G.W. Wong, R.L. Stevens, S.A. Krilis, Mast cells/basophils in the peripheral blood of allergic individuals who are HIV-1 susceptible due to their surface expression of CD4 and the chemokine receptors CCR3, CCR5, and CXCR4, Blood 97 (2001) 3484–3490.
[22] K. Suzuki, T. Shijuuku, T. Fukamachi, J. Zaunders, G. Guillemin, D. Cooper, A. Kelleher, Prolonged transcriptional silencing and CpG methylation induced by siRNAs targeted to the HIV-1 promoter region, J. RNAi Gene Silencing 1 (2005) 66–78.
[23] U. von Schwedler, J. Song, C. Aiken, D. Trono, Vif is crucial for human immunodeficiency virus type 1 proviral DNA synthesis in infected cells, J. Virol. 67 (1993) 4945–4955.
[24] R. Inubushi, R. Shimano, Y. Oshima, A. Adachi, The potential of various HIV-1 mutants to inhibit the replication of wild-type virus,, Biochem. Biophys. Res. Commun. 247 (1998) 349–352.
[25] L.P. Yang, J.L. Riley, R.G. Carroll, C.H. June, J. Hoxie, B.K. Patterson, Y. Ohshima, R.J. Hodes, G. Delespesse, Productive infection of neonatal CD8+ T lymphocytes by HIV-1, J. Exp. Med. 187 (1998) 1139–1144.
[26] Y. Yi, S. Rana, J.D. Turner, N. Gaddis, R.G. Collman, CXCR-4 is expressed by primary macrophages and supports CCR5-independent infection by dual-tropic but not T-tropic isolates of human immunodeficiency virus type 1, J. Virol. 72 (1998) 772–777.
[27] H.M. Naif, S. Li, M. Alali, A. Sloane, L. Wu, M. Kelly, G. Lynch, A. Lloyd, A.L. Cunningham, CCR5 expression correlates with susceptibility of maturing monocytes to human immunodeficiency virus type 1 infection, J. Virol. 72 (1998) 830–836.
[28] W. Abbas, G. Herbein, Plasma membrane signaling in HIV-1 infection, Biochim. Biophys. Acta 1838 (2014) 1132–1142.
[29] G.B. Melikyan, HIV entry: a game of hide-and-fuse? Curr. Opin. Virol. 4 (2014) 1–7.
[30] G.C. Carter, L. Bernstone, D. Sangani, J.W. Bee, T. Harder, W. James, HIV entry in macrophages is dependent on intact lipid rafts, Virology 386 (2009) 192–202.
[31] K.N. Venugopala, V. Rashmi, B. Odhav, Review on natural coumarin lead compounds for their pharmacological activity, Biomed. Res. Int. 2013 (2013) 963248.
[32] S.G. Deeks, A.N. Phillips, HIV infection, antiretroviral treatment, ageing, and non-AIDS related morbidity, BMJ 338 (2009) a3172.
[33] M.J. Silverberg, D.I. Abrams, AIDS-defining and non-AIDS-defining malignancies: cancer occurrence in the antiretroviral therapy era, Curr. Opin. Oncol. 19 (2007) 446–451.
[34] F. Bonnet, C. Burty, C. Lewden, D. Costagliola, T. May, V. Bouteloup, E. Rosenthal, E. Jougla, P. Cacoub, D. Salmon, G. Chene, P. Morlat, Changes in cancer mortality among HIV-infected patients: the Mortalite 2005 Survey, Clin. Infect. Dis. 48 (2009) 633–639.
[35] S. Okada, H. Goto, M. Yotsumoto, Current status of treatment for primary effusion lymphoma, Intractable Rare Dis. Res. 3 (2014) 65–74.
[36] J.P. Spano, D. Costagliola, C. Katlama, N. Mounier, E. Oksenhendler, D. Khayat, AIDS-related malignancies: state of the art and therapeutic challenges, J. Clin. Oncol. 26 (2008) 4834–4842.
[37] N. Mounier, C. Katlama, D. Costagliola, R.M. Chichmanian, J.P. Spano, Drug interactions between antineoplastic and antiretroviral therapies: implications and management for clinical practice, Crit. Rev. Oncol. Hematol. 72 (2009) 10–20.