Jikeikai Med J 2004 ; 51 : 25-31

Inhibitory Effect of $NMSO_3$ on Replication of Human Immunodeficiency Virus

Shogo NAKAMORI[1], Akira YOKONO[1], and Mariko NAKAMURA[2]

[1]*Solvay Seiyaku K.K.*
[2]*Department of Microbiology, The Jikei University School of Medicine*

ABSTRACT

The efficacy of $NMSO_3$, a synthetic derivative of various alkyl-sulfated sialic acid, against human immunodeficiency virus type 1 (HIV-1) was evaluated *in vitro*. In cultures of primary-infected human T-lymphoblastoid H9 cells, $NMSO_3$ inhibited HIV replication in a concentration-dependent manner. $NMSO_3$ also inhibited HIV in cultures of chronically infected cells. The production of progeny viruses was abolished without cytotoxicity by continuous addition of $NMSO_3$ to chronically infected H9 cells. Experiments to define the mechanism of inhibition showed that $NMSO_3$ competes with gp120 for binding to CD4 receptors and inhibits the entry of HIV into cells. Moreover, chloramphenicol acetyltransferase (CAT) analysis revealed that transcription of the integrated provirus is inactive in the presence of $NMSO_3$, suggesting the lack of progeny in the culture supernatant of $NMSO_3$-treated cells. These findings indicate that $NMSO_3$ has a unique mechanism of action against HIV replication and might be used to treat HIV infection.

(Jikeikai Med J 2004 ; 51 : 25-31)

Key words : human immunodeficiency virus, antiviral, CD4, transcription

INTRODUCTION

Since Acquired Immunodeficiency Syndrome was first described in 1981, there has been considerable research aimed at identifying compounds effective against Human Immunodeficiency Virus (HIV). Infection by HIV involves several distinct steps, including binding to CD4 and chemokine receptors, entry into a host cell, uncoating, reverse transcription, expression of the genome, protein synthesis, assembly, budding, and maturation.

HIV infects several subsets of cells, and infection by HIV is a chronic process characterized by persistent viral replication[1-4]. Several approaches have been explored to develop agents for treating HIV infection. Each step of viral replication is a possible target for therapeutic agents. Since the discovery of azidothymidine[5], many kinds of sugar-modified nucleoside analogs have been synthesized for their potential as anti-HIV compounds. Furthermore, several nonnucleoside reverse transcriptase (RT) inhibitors and protease inhibitors have used clinically. Although highly active antiretroviral therapy has been developed[6-8], more effective and less cytotoxic agents are still needed, particularly agents with different mechanisms of action. Because of extensive replication of HIV *in vivo*, resistance against HIV develops, eventually leading to treatment failure[9-13]. Therefore, other anti-HIV agents are needed.

$NMSO_3$ is a synthetic derivative of various alkyl-sulfated sialic acids which is effective against respiratory syncytial virus and other myxovirus infections[14].

Received for publication, January 27, 2004
中森　省吾，横野　　彰，中村真理子
Mailing address : Department of Microbiology, The Jikei University School of Medicine, 3-25-8, Nishi-Shimbashi, Minato-ku, Tokyo, 105-8461, Japan.
e-mail : nakamura@jikei.ac.jp

In the present study, we investigated the antiviral effect of $NMSO_3$ on HIV replication and analyzed the mechanism of its inhibitory action. We found that $NMSO_3$ prevents both the binding of virus to cells and the transcription of the viral genome. The identification of this novel class of anti-HIV compound and clarification of its mechanism of inhibition may lead to new therapies for HIV.

MATERIALS AND METHODS

Compound

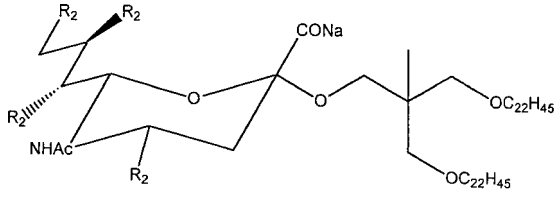
$NMSO_3$ was synthesized from alkyl-sulfated sialic acid at the Central Research Institute of Nissin Food Products Co., Ltd. (Shiga) with a method described by Fujita et al[15]. The chemical name of $NMSO_3$ is sodium [2, 2-bis (docosyl-oxymethyl) propyl-5-acetoamido-3, 5-dideoxyl-4, 7, 8, 9-tetra-O-(sodium-oxysulfonyl)-D-glycerol-α-D-galacto-2-nonulopyranosid] onate (Fig. 1).

Azidothymidine[5] was used as a positive anti-HIV control drug (Sigma Chemical Co., St. Louis, MO, USA).

Monoclonal antibody OKT4A was purchased from Beckton Dickinson (San Jose, USA).

Antiviral assay

Twenty microliters of H9 cells[16] (4×10^6 cells/ml) were incubated with 20 μl of serially diluted $NMSO_3$ for 30 minutes at 37°C. The cells were then inoculated with HIV-1 IIIB, MN, and clinical isolates 9487 and 9532. Reaction mixtures containing the cells, $NMSO_3$, and HIV were incubated for 90 minutes at 37°C to permit adsorption of viral particles, then were transferred to a new well of a 96-well plate containing 180 μl of fresh medium (10% fetal bovine serum/RPMI). On day 6, samples were harvested for 1) p24 enzyme-linked immunosorbent assay (ELISA) to quantify the number of progeny viruses, and 2) 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay[17] to evaluate the number of viable cells.



R2: OSO_3Na

Fig. 1. The structure of $NMSO_3$

To assess the effects of $NMSO_3$ on H9 cells chronically infected with HIV, the cells were infected with HIV IIIB at least 1 month before the experiment, and the percentage of infected cells was confirmed by fluorescence-activated cell sorter (FACS) analysis. The cells were seeded at 1×10^5/ml in a 24-well culture plate with 20 μg/ml of $NMSO_3$. Cells were split 1 : 5 every 3 to 4 days with addition of further $NMSO_3$ (final concentration, 20 μg/ml). The amount of produced viruses was monitored by measuring the activity of RT.

Competition analysis for recombinant gp120 binding to CD4

To determine the mechanism of action of $NMSO_3$ on HIV replication, we first examined the initial step of HIV infection, i.e., the binding of gp120 to the CD4 receptor. We performed a blocking assay with $NMSO_3$ to evaluate its interference activity against the binding between rgp120 and immobilized CD4.

Recombinant soluble CD4 (Intracel, Frederick, MD, USA) at 1.5 μg/ml was applied to an Immulon4 plate (Nunc Inter Med, Roskilde, Denmark) at 37°C for 1 hour. After blocking with 5% goat serum/phosphate-buffered saline at 37°C for 1 hour, serially diluted $NMSO_3$ with 150 ng of recombinant (r)gp120 was added to each well of a plate, which was incubated for an additional hour at room temperature. The rgp120 alone was reacted as the 100% binding control, and blocking buffer alone was reacted as the 0% binding control. One hundred microliters of rabbit anti-rgp120 serum was added, and the plate was incubated at room temperature for 1 hour. Horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G at 1 : 4,000 dilution was then added, and the plate was incubated at room temperature for 1 hour. Finally, ortho-phenylene diamine substrate

was added, and reactivity was measured by monitoring optical density at 492 nm and 630 nm.

Chloramphenicol acetyltransferase assay for HIV gene transcription

To investigate the mechanism of inhibition, we next analyzed the level of transcription by means of a chloramphenicol acetyltransferase (CAT) assay[18]. H9 cells were transfected with a plasmid containing the CAT gene under control of the HIV long terminal repeat (LTR) promoter. The LTR-driven CAT gene expression induced by *tat* was analyzed with $NMSO_3$ treatment. Five micrograms of LTR-CAT, which contains the CAT gene under control of HIV-1 LTR, and 5 μg of *ptat* (SV40-*tat* expression vector) plasmid (both kindly provided by Dr. Akio Adachi, Tokushima University School of Medicine)[19] were cotransfected into H9 cells (1.7×10^7 c/ml in 300 μl of 1 mM dextrose/RPMI) by Gene pulser electroporation (BioRad Laboratories, Hercules, CA, USA) The efficiency of transfection was approximately 30%. The cells were resuspended with 10% fetal bovine serum/RPMI, incubated for 48 hours in serially diluted $NMSO_3$, and harvested for CAT assay with ELISA (Boehringer Mannheim GmbH, Mannheim, Germany).

Results

Inhibitory effect against HIV primary infection

Treatment with $NMSO_3$ inhibited HIV-1 replication in a concentration-dependent manner in this primary assay system (Fig. 2). Little cytotoxicity was observed with $NMSO_3$ concentrations up to 250 μg/ml.

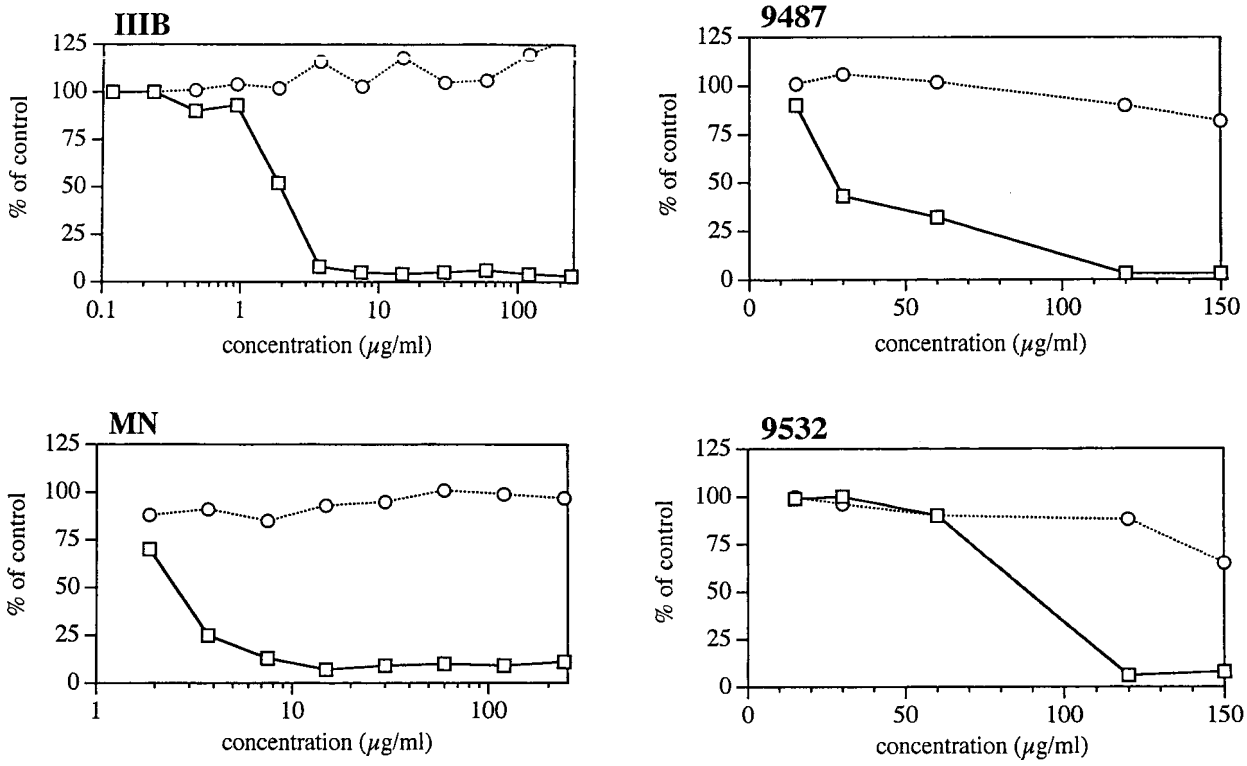


Fig. 2. Inhibitory effect of $NMSO_3$ on primary HIV infection
H9 cells were preincubated with various concentrations of $NMSO_3$ at 37°C for 30 minutes and exposed to HIV-1 IIIB, MN, and clinical isolates 9487 and 9532. The amounts of progeny viruses in culture supernatant were estimated with the p24 antigen capture assay, and the viability of cells was measured with the MTT assay on day 6. The values represent the percent of control (no agent). The results represent the average of triplicate experiments.
□: p24 antigen, ○: MTT

Anti-HIV activity in chronic infection

Treatment with $NMSO_3$ also had anti-HIV activity against H9 cells chronically infected with HIV-1 IIIB. Treatment with $NMSO_3$ completely abolished production of progeny viruses after 90 days. Viral replication was completely inhibited as long as $NMSO_3$ was present in the culture medium. However, no effect on cell viability was observed, and cultures without $NMSO_3$ showed continuous virus production during the experimental period. Furthermore, the effect of $NMSO_3$ on HIV replication was reversible: when treatment of $NMSO_3$ was halted, viral particles reappeared in the culture supernatant.

Competition analysis against CD4 receptors

$NMSO_3$ inhibited rgp120 binding to recombinant soluble CD4 in a concentration-dependent manner (Fig. 4 (A)). The result indicates that $NMSO_3$ can protect CD4 receptors from HIV binding. However, the control experiment with azidothymidine exhibited no inhibition. $NMSO_3$ also inhibited the binding of CD4 and OKT4A (Fig. 4B). In contrast, no inhibition

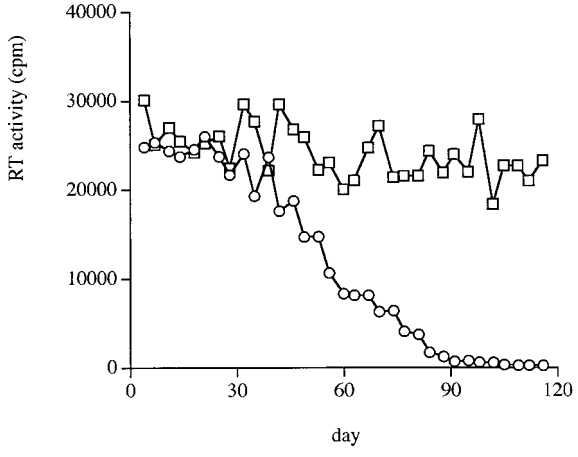


Fig. 3. Inhibition profile of $NMSO_3$ on HIV-1 chronically infected cells
$NMSO_3$ was added to HIV-1 IIIB-infected H9 cells, and the supernatant was harvested to measure the progeny viruses produced. The values indicate the incorporated [^{3}H] -deoxythymidine monophosphate, representing RT activity. The results represent the average of 2 separate experiments.
○: with $NMSO_3$, □: without $NMSO_3$

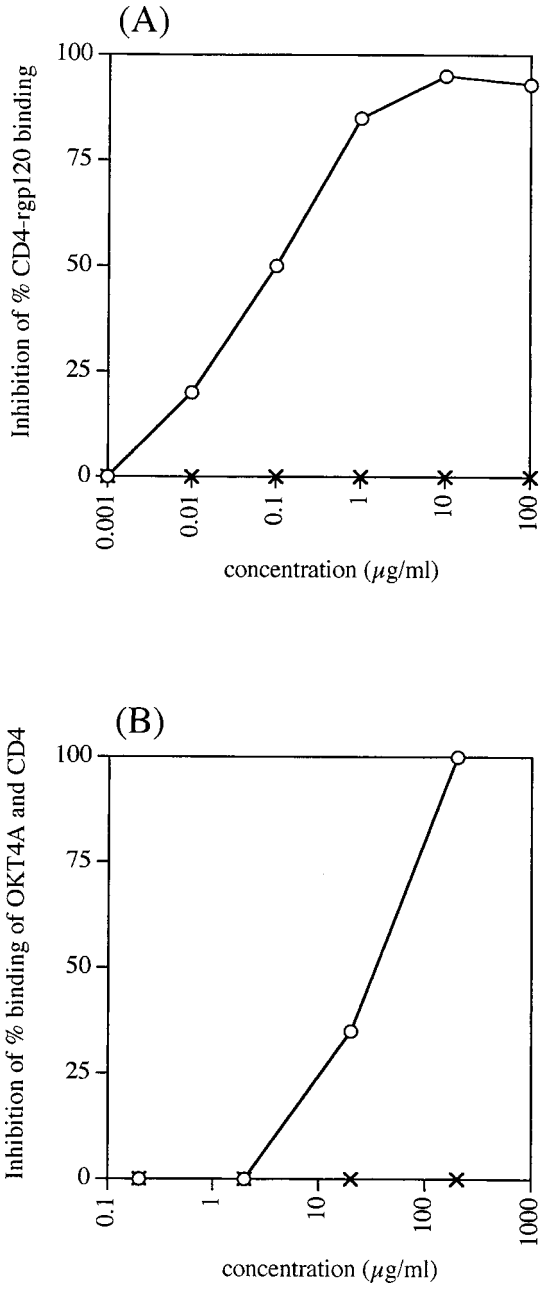


Fig. 4. Competition analysis with $NMSO_3$
A. Inhibition by $NMSO_3$ of the binding of rgp120 and CD4
Serially diluted $NMSO_3$ or azidothymidine was mixed with 150 ng of rgp120 and added to the soluble CD4-coated plate. The amount of bound rgp120 was determined with ELISA.
B. Inhibition by $NMSO_3$ of CD4 and OKT4A binding
Serially diluted $NMSO_3$ or azidothymidine was added to the soluble CD4-coated plate in the presence of OKT4A. The amount of bound OKT4A was determined with ELISA.
○: $NMSO_3$, ×: azidothymidine

was observed with azidothymidine.

Analysis of HIV gene transcription with $NMSO_3$

The LTR-driven CAT gene expression induced by *tat* was reduced by $NMSO_3$ in a concentration-dependent manner (Fig. 5). This result suggests that $NMSO_3$ treatment inactivated the transcription of HIV-1 proviruses.

Discussion

A variety of potentially vulnerable steps in the HIV replication cycle could serve as targets for therapeutic intervention, and advances in the understanding of the HIV replication cycle have identified molecules that can be selectively inhibited[20]. Although several promising compounds have been developed and highly active antiretroviral therapy has made significant progress[21], more effective agents are still needed, in particular, agents with different mechanisms of action.

$NMSO_3$, a sulfated sialyl lipid, has been evaluated for efficacy against respiratory syncytial virus and other myxovirus infections in cell culture[14]. In an experiment with a temperature shift during the period of contact between the virus and cells, $NMSO_3$ inhibited both the binding of respiratory syncytial virus to the cells and its penetration into the cells by means of its high anionic charge[14]. In the present study, we investigated the inhibitory activity of $NMSO_3$ against HIV-1. Our results indicate that $NMSO_3$ inhibits HIV replication in both acute and chronic infections.

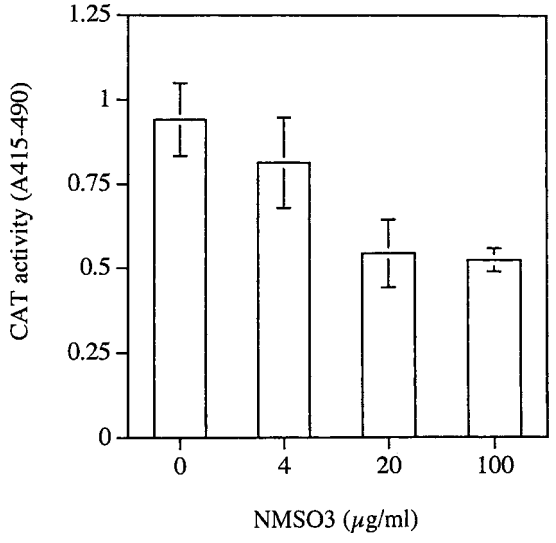


Fig. 5. Inhibition of HIV-1 LTR-driven CAT gene expression
H9 cells were cotransfected with HIV-1 LTR-CAT and *ptat* by electroporation and treated with $NMSO_3$. To examine the level of expression after 48 hours, a CAT antigen capture assay was performed.

Competition ELISA between CD4 and gp120 has shown that $NMSO_3$ selectively blocks CD4/gp120 binding. The first step of HIV infection is the binding of gp120 to CD4 molecules on the cell surface[22]; therefore, molecules that block this interaction could inhibit both HIV infection and cell fusion[23-25]. Previous studies have shown that dextran sulfate binds to the gp120 through its high anionic charge and blocks the fusion process of HIV into cells[26]. $NMSO_3$ has a structure similar to that of dextran sulfate, i.e., a highly anionic charged polysulfate structure. Moreover, negatively charged polysaccharides inhibit the cellular adsorption of viruses by interfering with static electrical binding between the viral envelope and cell membranes[27]. $NMSO_3$ has 4 sulfate residues per molecule, is negatively charged, and may inhibit the binding of HIV gp120 to CD4 on cells[28]. Therefore, $NMSO_3$ interacts with CD4 and in primary infection likely inhibits the binding of gp120 to CD4, which terminates viral entry into cells.

CD4 has 4 extracellular domains, of which the D1 domain contains 3 CDR-like regions[29,30]. Competition analysis has identified the region with which $NMSO_3$ interacts. OKT4A, which recognizes the CDR2-like region, competes strongly with $NMSO_3$. CDR2 is a gp120-binding site[31], and OKT4A competes with HIV to bind to CD4[32]. These findings suggest that the anti-HIV activity of $NMSO_3$ depends on the interaction of gp120 with CD4 receptors, especially the D1 domain of CD4.

We next attempted to determine the stage at which the HIV-1 replication cycle is blocked following cell surface interaction between $NMSO_3$ and the CD4 receptor, because $NMSO_3$ also inhibits the production of progeny viruses from cells chronically infected with HIV-1. To investigate the inhibitory

effect during chronic infection, we examined viral expression in the presence of $NMSO_3$. The LTR-driven CAT assay showed inhibition of viral transcription. Benkirane et al. have reported that the monoclonal antibody 13B8-2, which is specific for the CDR3-like region in domain 1 of CD4, inhibits HIV-1 provirus transcription[33], that the cytoplasmic tail of CD4 is required for that inhibition[34], and that 13B8-2 inhibits the activation of mitogen-activated protein kinase induced in HIV-infected cells[35]. Therefore, we believe that the mechanism of action of $NMSO_3$ is similar to that of 13B8-2 and that the inhibition of HIV transcription corresponds to the inactivation of signaling pathways participating in the regulation of HIV progeny production.

The anti-HIV activity of $NMSO_3$ we have described suggests that $NMSO_3$ may be useful for treating HIV infection in both the primary and chronic periods. Moreover, a combination of chemotherapic agents may enhance the efficacy and reduce the cytotoxicity of individual compounds[36,37]. Therefore, $NMSO_3$, which induces translational arrest, might be used in combination therapy with nucleoside analogues, such as azidothymidine and dideoxyinosine (ddI)[38].

In the view of the selectivity of $NMSO_3$ as an inhibitor of HIV-1 replication *in vitro*, further evidence for its potential use in therapy is needed. Furthermore, examination of this $NMSO_3$'s effects on the HIV replication cycle helps clarify the mechanism of HIV multiplication.

Acknowledgement : We would like to thank Prof. Donald W. Kufe, Harvard Medical School, for his critical reading of this manuscript.

This research was supported by a grant of "Bio-Venture Research Fund Project Aid" from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

REFERENCES

1. Peterson A, Seed B. Genetic analysis of monoclonal antibody and HIV binding sites of the human lymphocyte antigen CD4. Cell 1988 ; 54 : 65-72.
2. Strain MC, Gunthard HF, Havlir DV, Ignacio CC, Smith DM, Leigh-Brown AJ, et al. Heterogeneous clearance rates of long-lived lymphocytes infected with HIV : intrinsic stability predicts lifelong persistence. Proc Natl Acad Sci USA 2003 ; 100 : 4819-24.
3. Chun TW, Fauci AS. Latent reservoirs of HIV : obstacles to the eradication of virus. Proc Natl Acad Sci USA 1999 ; 96 : 10958-61.
4. Grossman Z, Feinberg MB, Paul WE. Multiple modes of cellular activation and virus transmission in HIV infection : a role for chronically and latently infected cells in sustaining viral replication. Proc Natl Acad Sci USA 1998 ; 95 : 6314-9.
5. Mitsuya H, Weinhold KJ, Furman PA, St Clair MH, Lehrman SN, Gallo RC, et al. 3′-Azido-3′-deoxythymidine (BW A509U) : an antiviral agent that inhibits the infectivity and cytopathic effect of human T-lymphotropic virus type III/lymphadenopathy-associated virus in vitro. Proc Natl Acad Sci USA 1985 ; 82 : 7096-100.
6. Carpenter CC, Cooper DA, Fischl MA, Gatell JM, Gazzard BG, Hammer S-M et al. Antiretroviral therapy in adults : updated recommendations of the International AIDS Society-USA Panel. JAMA 2000 ; 283 : 381-90.
7. Collier AC, Coombs RW, Schoenfeld DA, Bassett RL, Timpone J, Baruch A, et al. Treatment of human immunodeficiency virus infection with saquinavir, zidovudine, and zalcitabine. AIDS Clinical Trials Group. N Engl J Med 1996 ; 334 : 1011-7.
8. Staszewski S, Morales-Ramirez J, Tashima KT, Rachlis A, Skiest D, Stanford J, et al. Efavirenz plus zidovudine and lamivudine, efavirenz plus indinavir, and indinavir plus zidovudine and lamivudine in the treatment of HIV-1 infection in adults. N Engl J Med 1999 ; 341 : 1865-73.
9. Mansky LM, Pearl DK, Gajary LC. Combination of drugs and drug-resistant reverse transcriptase results in a multiplicative increase of human immunodeficiency virus type 1 mutant frequencies. J Virol 2002 ; 76 : 9253-9.
10. Martinez-Picado J, DePasquale MP, Kartsonis N, Hanna GJ, Wong J, Finzi D, et al. Antiretroviral resistance during successful therapy of HIV type 1 infection. Proc Natl Acad Sci USA 2000 ; 97 : 10948-53.
11. Sarmati L, Nicastri E, Uccella I, d'Ettorre G, Parisi SG, Palmisano L, et al. Drug-associated resistance mutations in plasma and peripheral blood mononuclear cells of human immunodeficiency virus type 1-infected patients for whom highly active antiretroviral therapy is failing. J Clin Microbiol 2003 ; 41 : 1760-2.
12. Frenkel LM, Wang Y, Learn GH, McKernan JL, Ellis GM, Mohan KM, et al. Multiple viral genetic analyses detect low-level human immunodeficiency virus type 1 replication during effective highly active antiretroviral therapy. J Virol 2003 ; 77 : 5721-30.
13. Gunthard HF, Frost SD, Leigh-Brown AJ, Ignacio CC,

Kee K, Perelson AS, et al. Evolution of envelope sequences of human immunodeficiency virus type 1 in cellular reservoirs in the setting of potent antiviral therapy. J Virol 1999 ; 73 : 9404-12.
14. Kimura K, Mori S, Tomita K, Ohno K, Takahashi K, Shigeta S, et al. Antiviral activity of $NMSO_3$ against respiratory syncytial virus infection *in vitro* and *in vivo*. Antiviral Res 2000 ; 47 : 41-51.
15. Fujita S, Numata M, Suzuki K, Nunomura S, Sugimoto M, Terada M. Novel compounds having anti-viral activity. Japanese Patent Application PCT/JP97/01654, MO97/43296. 1997.
16. Popovic M, Sarngadharan MG, Read E, Gallo RC. Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. Science 1984 ; 224 : 497-550.
17. Twentyman PR, Luscombe M. A study of some variables in a tetrazolium dye (MTT) based assay for cell growth and chemotherapy. Br J Cancer 1987 ; 56 : 279-85.
18. Shaw WV. Chloramphenicol acetyltransferase : enzymology and molecular biology. CRC Crit Rev Biochem 1983 ; 14 : 1-46.
19. Sakai H, Shibata R, Miura T, Hayami M, Ogawa K, Kiyomasu T, et al. Complementation of the rev gene mutation among human and simian lentiviruses. J Virol 1990 ; 64 : 2202-7.
20. De Clercq E. New anti-HIV agents and targets. Med Res Rev 2002 ; 22 : 531-65.
21. Debul M, Chun TW, Yoder C, Hidalgo B, Belson M, Hertogs K, et al. Short-cycle structures intermittent treatment of chronic HIV infection with highly active antiretroviral therapy : effects on virologic, immunologic, and toxicity parameters. Proc Natl Acad Sci USA 2001 ; 98 : 15161-6.
22. Fontenot JD, Tan X, Phillips DM. Structure-based design of peptides that recognize the CD4 binding domain of HIV-1 gp120. AIDS 1998 ; 12 : 1413-8.
23. Hussey RE, Richardson NE, Kowalski M, Brown NR, Chang HC, Siliciano RF, et al. A soluble CD4 protein selectively inhibits HIV replication and syncytium formation. Nature 1988 ; 331 : 78-81.
24. Arthos J, Deen KC, Chaikin MA, Fornwald JA, Sathe G, Sattentau QJ, et al. Identification of the residues in human CD4 critical for the binding of HIV. Cell 1989 ; 57 : 469-81.
25. Deen KC, McDougal JS, Inacker R, Folena-Wasserman G, Arthos J, Rosenberg J, et al. A soluble form of CD4 (T4) protein inhibits AIDS virus infection. Nature 1988 ; 331 : 82-4.
26. Moulard M, Lortat-Jacob H, Mondor I, Roca G, Wyatt R, Sodroski J, et al. Selective interactions of polyanions with basic surfaces on human immunodeficiency virus type 1 gp120. J Virol 2000 ; 74 : 1948-60.
27. Mastromarino P, Petruzziollo R, Macchia S, Rieti S, Nicoletti R, Orsi N. Antiviral activity of natural and semisynthetic polysaccharides on the early steps of rubella virus infection. J Antimicrob Chemother 1997 ; 39 : 339-45.
28. Kwong PD, Wyatt R, Sattentau QJ, Sodroski J, Hendrickson WA. Oligomeric modeling and electrostatic analysis of the gp120 envelope glycoprotein of human immunodeficiency virus. J Virol 2000 ; 74 : 1961-72.
29. Peterson A, Seed B. Genetic analysis of monoclonal antibody and HIV binding sites of the human lymphocyte antigen CD4. Cell 1988 ; 54 : 65-72.
30. Manning TC, Parke EA, Teyton L, Kranz DM. Effects of complementarity determining region mutations on the affinity of an alpha/beta T cell receptor : measuring the energy associated with CD4/CD8 repertoire skewing. J Exp Med 1999 ; 189 : 461-70.
31. Clayton LK, Sieh M, Pious DA, Reinherz EL. Identification of human CD4 residues affecting class II MHC versus HIV-1 gp120 binding. Nature 1989 ; 339 : 548-51.
32. Arthos J, Deen KC, Shatzman A, Truneh A, Rosenberg M, Sweet RW. The genetic analysis of the HIV envelope binding domain on CD4. Ann NY Acad Sci 1990 ; 616 : 116-24.
33. Benkirane M, Corboeau P, Housset V, Devaux C. An antibody that binds the immunoglobulin CDR3-like region of the CD4 molecule inhibits provirus transcription in HIV-1 infected T cells. EMBO J 1993 ; 12 : 4909-21.
34. Benkirane M, Schmid-Antomarchi H, Littman DR, Hirn M, Rossi B, Devaux C. The cytoplasmic tail of the CD4 molecule is required for inhibition of human immunodeficiency virus type 1 replication by antibodies that bind the CDR3-like region of the D1 domain of CD4. J Virol 1995 ; 69 : 6904-10.
35. Briant L, Signoret N, Gaubin M, Robert-Hebmann V, Zhang X, Murali R, et al. Transduction of activation signal that follows HIV-1 binding to CD4 and CD4 dimerization involves the immunoglobulin CDR3-like region in domain 1 of CD4. J Biol Chem 1997 ; 272 : 19441-50.
36. Guidelines for the Use of Antiretroviral Agents in HIV-Infected Adults and Adolescents. Department of Health and Human Services (DHHS) ; 2002.
37. Yarchoan R, Pluda JM, Thomas RV, Mitsuya H, Brouwers P, Wyvill KM, et al. Long-term toxicity/activity profile of 2′, 3′-dideoxyinosine in AIDS or AIDS-related complex. Lancet 1990 ; 336 : 526-9.
38. Gao WY, Zhou BS, Johns DG, Mitsuya H, Yen Y. Role of the M2 subunit of ribonucleotide reductase in regulation by hydroxyurea of the activity of the anti-HIV-1 agent 2′, 3′-dideoxyinosine. Biochem Pharmacol 1998 ; 56 : 105-12.