Viral infectious disease and natural products with antiviral activity

Kaio Kitazato[1], Yifei Wang[2], Nobuyuki Kobayashi[1, 3, *]

[1] *Division of Molecular Pharmacology of Infectious Agents, Department of Molecular Microbiology and Immunology, Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki, Japan;*
[2] *Guangzhoujinan Biomedicine Research & Development Center, Jinan University, Guangzhou, China;*
[3] *Central Research Center, AVSS. Co., 1-22 Wakaba-machi, Nagasaki, Japan.*

ABSTRACT: Viral diseases, such as acquired immunodeficiency syndrome (AIDS), respiratory diseases, and hepatitis, are the leading causes of death in humans worldwide, despite the tremendous progress in human medicine. The lack of effective therapies and/or vaccines for several viral infections, and the rapid emergence of new drug-resistant viruses have urged a growing need for developing new and effective chemotherapeutic agents to treat viral diseases. Recent advances in the understanding of both the cellular and molecular mechanisms of virus replication have provided the basis for novel therapeutic strategies. Several hundred natural products have been isolated for screening and identifying antiviral activity, and some have been shown to have great medicinal value in preventing and/or ameliorating viral diseases in preclinical and clinical trials. There are innumerable potentially useful medicinal plants and herbs waiting to be evaluated and exploited for therapeutic applications against genetically and functionally diverse virus families. This review focuses on several selected pathogenic viruses, including the human immunodeficiency virus (HIV), influenza virus, hepatitis B and C viruses and herpes viruses, and antiviral natural compounds from medicinal plants (herbs), while paying particular attention to promising compounds in preclinical and clinical trials. We also focused our attention on the need to develop effective screening systems for antiviral activity.

Key Words: HIV-1, influenza virus, HBV/HCV, HSV-1, HSV-2, antiviral, natural product, herbs, medicinal plant

**Correspondence to:* Division of Molecular Pharmacology of Infectious Agents, Department of Molecular Microbiology and Immunology, Graduate School of Biomedical Sciences, Nagasaki University, 1-14, Bunkyo-machi, Nagasaki 852-8521, Japan;
e-mail: nobnob@net.nagasaki-u.ac.jp

Received June 25, 2007
Accepted July 1, 2007

Introduction

Viral diseases, caused by pathogenic virus infections which have high morbidity and mortality rates, are still the leading cause of death in humans worldwide. Although effective vaccines have led or might lead to the eradication of important viral pathogens, such as smallpox, polio, and mumps, other viral diseases, such as human immunodeficiency virus (HIV) and hepatitis C virus (HCV), have proven difficult to combat using the conventional vaccine approach. Moreover, the emergence of viral resistance to drugs, as well as the serious adverse effects induced by antiviral drugs, has caused serious medical problems, particularly when administered in combination over prolonged treatment periods. Although many new antiviral drugs have been approved in recent years, most of them are used for the treatment of HIV, and these drugs are quite costly, thus limiting their use in developing countries, where infection is most prevalent.

A virus is a unique pathogen which is incapable of replicating without a host cell. It utilizes the host cell environment and cellular factors for its propagation. This unique feature of viruses makes it difficult to design a treatment to attack the virus or its replication directly without any adverse effects on the infected cells. However, viruses share a common stage in their replication cycle, which includes attachment and entry to the host cell, transcription of viral mRNA, replication of viral genome, assembly and budding as progeny virus particles, regardless of different genetic materials (DNA or RNA), or whether has a different invasion strategy of which enveloped with a lipid-containing membrane (enveloped virus) or not. Whereas, viruses with an RNA genome, such as HIV, HCV, and influenza, are genetically highly variable, due to the fact that viral reverse transcriptase or RNA-dependent RNA polymerase lack a proofreading mechanism. Accumulated mutations in viral RNA genome have been proven to be associated with the emergence of drug-resistant viruses (*1-3*). The emergence of drug-

resistant viruses presents a challenge for the design of new drugs. These problems emphasize the need to develop new antiviral drugs targeting different steps in the viral replication cycle.

An understanding of the molecular mechanisms of viral invasion and replication enables us to design antiviral drugs targeting the different stages of the viral replication cycle. Although in theory, any viral molecule that is essential for viral replication is a potential drug target, most of the clinically useful antiviral drugs are the molecules that can specifically target a single viral enzyme, which is crucial in viral replication (*4*). Targeting virus molecules is likely more specific, and less toxic. However, there is a narrow spectrum of viruses and a higher risk of creating resistant viruses. Whereas drugs which target cellular molecules may possess a broader antiviral activity spectrum and less risk of developing virus resistance, but may be more toxic to the host cell. Ideally, effective therapeutic agents that target multiple stages in the viral replication cycle with combined approaches but with little or no toxicity are desirable.

Traditional medicines, such as Chinese medicine (CM), have long been used as multiple combinations of compounds in the form of processed natural products. Medicinal herbs relieve the symptoms of many different human diseases, including infectious diseases, and have been used for thousands of years. CM is typically orally administered as hot-water extracts, which can be used for the prophylactic and therapeutic treatment of viral infections. A wide variety of natural compounds derived from medicinal plants (herbs) have been extensively studied in terms of their antiviral activity. Several hundred natural active compounds have been identified worldwide (*5-9*). Many of them have complementary and overlapping mechanisms of action, either inhibiting viral replication, or synthesis of the viral genome. These natural active compounds, which contain more characteristics of high chemical diversity and biochemical specificity than standard combinatorial chemistry, offer major opportunities for finding novel lead structures that are active against a wide range of assay targets. In addition, natural products that are biologically active in assays are generally small molecules with drug-like properties. Namely, they are capable of being absorbed and metabolized by the body. Hence, the development costs of producing orally active medicines are likely to be much lower than that of biotechnological products or most compounds produced to date from combinatorial chemistry. Therefore, natural products, including traditional medicinal plants (herbs), offer great promise as potentially effective new antiviral drugs.

Selected viral diseases and antiviral agents

HIV/AIDS and antiviral agents

Human immunodeficiency virus (HIV) is the causative pathogen of acquired immunodeficiency syndrome (AIDS). HIV has cumulatively infected over 60 million individuals and caused the deaths of over 28 million people since it was first recognized in 1981, and is the most destructive epidemic of recent times. This global epidemic remains out of control, and transmission is rapidly spreading worldwide. According to the WHO 2006 global summary of the AIDS epidemic, the number of people living with HIV continues to grow; a total of 39.5 million people were living with HIV, a total of 4.3 million people were newly infected with HIV, and a total of 2.9 million people died due to AIDS in 2006. The majority of people living with HIV are in developing countries, such as sub-Saharan Africa and East and South Asia (*10*). Induction of the highly active antiretroviral therapy (HAART), a combination therapy with reverse transcriptase and protease inhibitors, has significantly improved the clinical outcome of HIV infection and AIDS, greatly reduced morbidity and mortality in HIV-1-infected individuals, and dramatically improved the life expectancy of AIDS patients. However, the treatment cannot eradicate the virus from infected individuals and is quite often limited by the emergence of drug-resistant HIV-1 strains and long-term toxicity. In addition, the high cost of anti-HIV drugs limits the ability of HIV-infected people and AIDS patients in developing countries to access HARRT (*11*). The discovery of low cost, effective medicinal agents is therefore urgently needed.

HIV-1 is unique in terms of its transmission and replication. HIV-1 is transmitted both by sexual contact and heamatogenously through contaminated needles or blood products, so the virus can initiate infection by crossing a mucosal barrier or by direct entry into a T cell or monocyte/macrophage lineage cell in the peripheral blood. HIV-1 can spread after a long latent period of infection. Recent advances in the understanding of the cellular and molecular mechanisms of HIV-1 entry and replication have provided the basis for novel therapeutic strategies to prevent viral penetration of the target cell-membrane and inhibit virus multiplication (Figure 1). HIV-1 entry into host cells represents a complex sequence of events involving several viral and cellular proteins that are potential targets for drug development. Targeting the host cell factors involved in the regulation of HIV-1 replication might be one way to overcome the resistance of HIV-1 to antiviral drugs. Both CXCR4 and CCR5 chemokine receptors are co-receptors for HIV entry to the host cell, and their antagonists are being investigated as HIV entry inhibitors in controlled clinical trials (*12*). The inhibitors will be needed in combination in order to inhibit viral replication, and even in combinations of antiviral drugs that also target other aspects of the HIV replication cycle, such as reverse transcriptase and protease, to obtain optimum therapeutic effects. Currently, the number of anti-

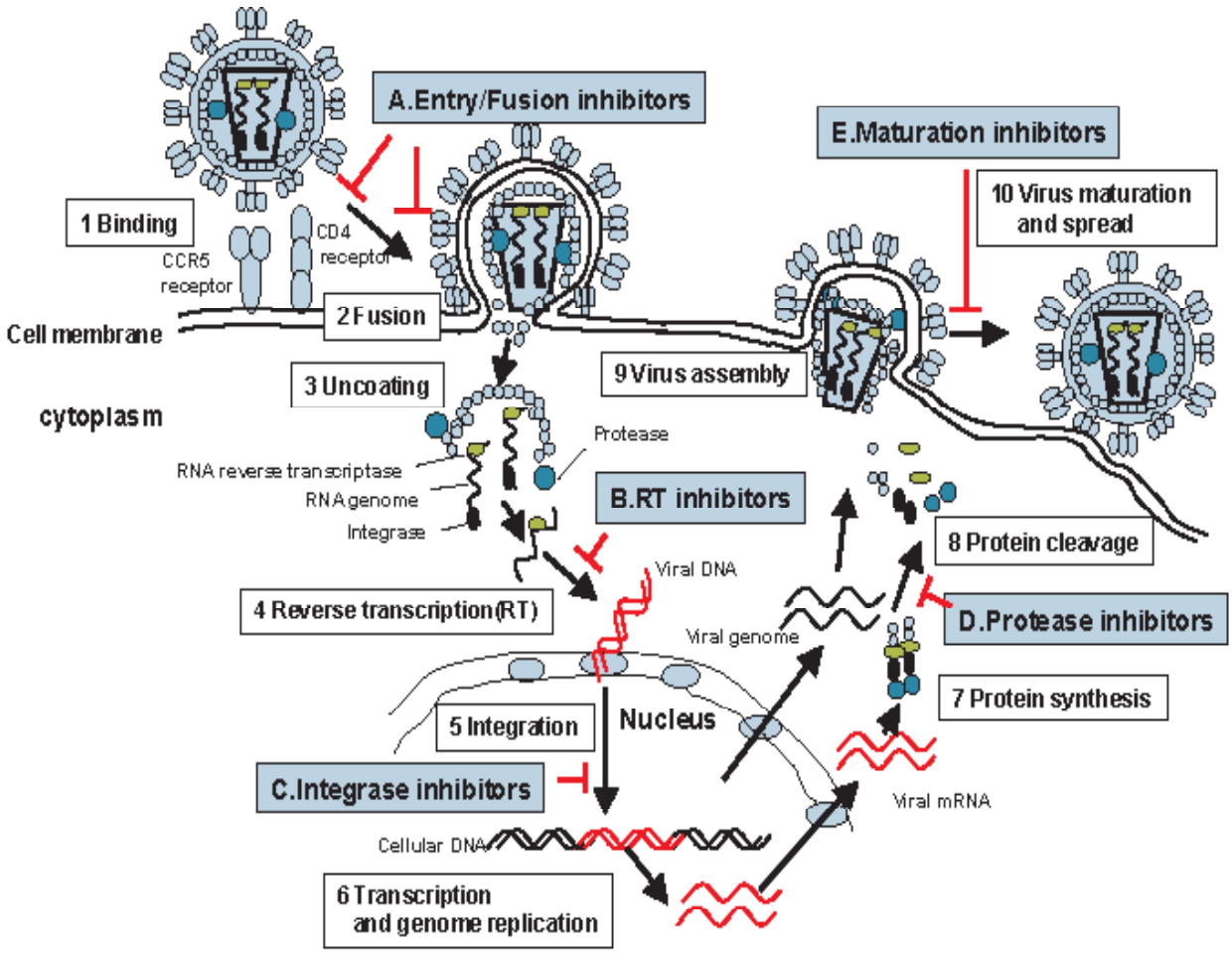

Figure 1. The HIV-1 replication life cycle. The replication life cycle of human immunodeficiency virus (HIV) have several specific steps (1-10), many of which are targets for antiviral drugs (A-E).

HIV/AIDS therapeutic drugs approved by the FDA has increased to 26 drugs from the first approved drug, AZT, in 1987 (*13*). These anti-HIV/AIDS drugs can be categorized into fusion inhibitors (FIs), nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), nonnucleoside reverse transcriptase inhibitors (NNRTIs), and protease inhibitors (PIs) (*14-17*). However, HIV-1 has developed an extraordinary degree of genetic diversity. To date, a high number of mutations in protease, reverse transcriptase, and gp41 have been associated with reduced susceptibility to the antiretroviral drugs currently available. In addition, new integrase inhibitors and maturation inhibitors that target Gag have shown promising effects in preclinical and clinical trials (*17-20*).

Substantial progress has been made on the use of natural products as anti-HIV agents, and several natural products, mostly of plant origin, have been shown to possess promising activities that could assist in the prevention and/or amelioration of the disease. Table 1 summarizes some major natural compounds with anti-HIV activity derived from plants or herbs (*19-35*). The following natural products from plant origin have been cited as promising anti-HIV agents: Betulinic acid (a pentacyclic triterpene) isolated from the bark of the white birch tree, has been demonstrated to inhibit maturation of the HIV-1 Gag precursor assembled *in vitro* (*23-26*), Chinese herbal medicine, *Scutellaria baicalensis* Georgi and its identified components, Baicalin (a flavonoid), calanolides (coumarins), have been shown to inhibit infectivity and replication of HIV (*17,28-35*). Flavonoids inhibit HIV-1 activation via a novel mechanism, and these agents are potential candidates for therapeutic strategies aimed at maintaining a cellular state of HIV-1 latency. Acute HIV-1 infection has been shown to be suppressed by certain flavonoids *in vitro*, and evidence for inhibition of HIV-1 protease, integrase, and reverse transcriptase by flavonoids also exists (*28-32*). Calanolide A, a coumarins, is a potent non-nucleoside reverse transcriptase inhibitor (NNRTI) of human immunodeficiency virus type 1 (HIV-1) (*33-35*), recently discovered in extracts from the tropical rainforest tree, *Calophyllum lanigerum*. Recent studies have also shown that several polysaccharides are effective inhibitors of HIV replication. Some of the presented compounds demonstrated *in vitro* synergism; thus there is the rationale of their combined use in HIV-infected individuals (*9,26,34*). Although extensive research has been performed to assess both

Table 1. Natural products with anti-HIV activity

Compounds	Origin of plant	Activity/Target	References
Terpenoids			
Agastanol and Agastaquinone	*Agastache rugosa*	Protease	*19*
Uvaol and Ursolic acid	*Crataegus pinnatifida*	Protease	*20*
Garciosaterpene A,C	*Garcinia speciosa*	Reverse transcriptase Inhibition in syncytium	*21*
Vaticinone	*Vatica cinerea*	Inhibited replication	*22*
Betulinic acid	Widely distributed	Inhibited maturation	*23-26*
Glycyrrhizin	*Glycyeehiza spp.*	Inhibited Infectivity, cytopathic activity, replication	*27*
Flavonoids			
Baicalin	*Scutellaria baicalensis*	Reverse transcriptase infection/entry, replication	*28* *29*
Taxifolin (dihydroquercetin)	*Juglans mandshurica*	Inhibited cytopathic activity	*30*
(-)-Epigallocatechin-3-gallate (EGCG)	Green tea	Reverse transcriptase	*31*
Flavonoid glucuronide	*Charysanthemum morifolium*	Integrase	*32*
Coumarins			
Calanolide A	*Calophyllum lanigerum*	Reverse transcriptase	*33-35*

the beneficial effects and the risks of herbal medicines in patients with HIV infection and AIDS, the potential beneficial effects need to be confirmed in large, rigorous trials. There is insufficient evidence to support the use of herbal medicines in HIV-infected individuals and AIDS patients.

Influenza virus and antiviral agents

Influenza A virus is one of the most common infectious pathogens in humans. It is a seasonal, acute, highly transmissible respiratory disease. Influenza in humans is caused by two subtypes of influenza virus A and B. Influenza virus A mutates easily, thereby often causing new antigenic variants of each subtype to emerge. The threat of a human influenza pandemic has greatly increased over the past several years with the emergence and continuing global spread of the highly pathogenic avian influenza viruses, notably H5N1 virus. The current widespread circulation of H5N1 viruses among avian populations in several Asian, African and European countries and the transmission from avian species to humans with a high mortality rate of more than 50%, warn us to prepare for the next pandemic threat.

The control and treatment of influenza depends mainly on chemical and biochemical agents. There are two classes of anti-influenza drugs currently available for influenza therapy, which target either the influenza A M2 ion channel or neuraminidase (NA) (*36*). However, the emergence of resistance to these drugs has been detected, which raises concerns regarding their widespread use (*37*). The viral particles have two surface antigens, haemagglutinin and sialidase (neuraminidase), that decorate the surface of the virus and have been implicated in viral attachment and fusion, and the release of virion progeny, respectively. The receptor for haemagglutinin is the terminal sialic acid residue of the host cell surface sialyloligosaccharides, while sialidase catalyses the hydrolysis of terminal sialic acid residues from sialyloligosaccharides. The enzyme neuraminidase (NA) is an attractive target for antiviral strategy because of its essential role in the pathogenicity of many respiratory viruses. NA removes sialic acid from the surface of infected cells and virus particles, thereby preventing viral self-aggregation and promoting efficient viral spread; NA also plays a role in the initial penetration of the mucosal lining of the respiratory tract. Since the influenza virus genome does not have the processing protease for the viral membrane fusion glycoprotein precursors, entry of this virus into cells is determined primarily by host cellular, trypsin-like proteases that proteolytically activate the fusion glycoprotein precursors of Influenza A virus. The protease determine the infectious organ tropism of Influenza A virus infection as well as the efficiency of viral multiplication in the airway. Administration of protease inhibitors in the early-stage of infection significantly suppresses viral entry and viral multiplication (*38*).

To date, some of the anti-influenza agents that have been isolated from plants include a variety of polyphenols, flavonoids, and alkaloids (*39*), summarized in Table 2. Polyphenol-rich extract from the medicinal plant *Geranium sanguineum* L. has been reported to show a strong anti-influenza virus activity, as well as antioxidant and radical scavenging capacities (*40*). A biflavonoids, ginkgetin isolated from *Ginkgo biloba* L. and *Cephalotaxus harringtonia* K. Koch show a potent inhibitory activity against influenza virus sialidase (*41*,*42*). A combination of NA inhibitors and protease inhibitors could be potentially used as a potent anti-influenza therapy in order to minimize the emergence of drug-resistant mutant viruses. Although most clinical trials have reported some benefits from the use of antiviral herbal medicines, there remains a need for larger, stringently designed, randomized clinical trials to provide conclusive evidence of their efficacy. An indole alkaloid from *Uncaria rhynchophylla* and the pavine alkaloid (-)-thalimonie (Th1) from *Thalictrum*

Table 2. Natural products with anti-influenza activity

Compounds	Origin of plant	Activity/Target	References
Polyphenols			
Polyphenolic complex	*Geranium sanguineum* L.	Influenza virus	*40*
Flavonoids			
biflavonoids (Ginkgetin)	*Ginkgo biloba* L.	influenza virus sialidase	*41*
tetrahydroxyflavone	*Scutellaria baicalensis*	influenza virus sialidase	*42*
Alkaloids			
Thalimonine	*Thalictrum simplex* L.	influenza virus replication	*43*
Indole alkaloid	*Uncaria rhynchophylla*	influenza virus replication	*44*
Lignans			
rhinacanthin E,F	*Rhinacanthus nasutus*	Influenza virus	*45*

simplex also exhibit potent inhibitory effects against influenza A viruses (*43*). Many medicinal plants (herbs) including the *Bergenia ligulata*, *Nerium indicum* and *Holoptelia integrifolia* plants, exhibit considerable antiviral activities against the influenza virus (*44*). Furthermore, two new lignans with activity against the influenza virus from the medicinal plant *Rhinacanthus nasutus* have also been reported (*45*).

Hepatitis B and C viruses and antiviral agents

Hepatitis B virus (HBV) infection is a serious global health problem. According to WHO estimation, of the 2 billion people who have been infected with the hepatitis B virus (HBV), more than 350 million suffer from chronic HBV infection. These chronically infected persons are at a high risk of death from cirrhosis of the liver and liver cancer, diseases that kill about one million persons each year (*46,47*). On the other hand, hepatitis C virus (HCV) is a major cause of acute hepatitis and chronic liver disease, including cirrhosis and liver cancer. Globally, an estimated 170 million persons are chronically infected with HCV and 3 to 4 million persons are newly infected each year. HCV spreads primarily by direct contact with human blood. The major causes of HCV infection worldwide are use of unscreened blood transfusions, and re-use of needles and syringes that have not been adequately sterilized. No vaccine is currently available to prevent hepatitis C (*48,49*). Liver disease due to chronic HBV and HCV infection is becoming a leading cause of death among persons with HIV infection worldwide (*50*).

Currently available Anti-HBV and HCV drugs include interferon, lamivudine and ribavirin. The therapeutic effects of interferon for HBV are around 30%. The effect of interferon for HCV is 20%~30%. A combination therapy of interferon and ribavirin for HCV can increase the therapeutic efficacy up to 50%, but has serious side effects and can be prohibitively expensive for low-income countries with a high prevalence of HCV. Lamivudine inhibits HBV multiplication and significantly decreases the viral load, but can easily induce resistance (*51*). Many patients who use natural products, including those who are not eligible for IFN/ribavirin, cannot afford treatment, or fail to respond to IFN.

Oxymatrine and matrine are the two major alkaloid components found in sophora roots. They are obtained primarily from the above ground portion of *Sophora alopecuroides* L., *Sophora flavescens* and *Sophora subprostrata* (shandougen). An intensive investigation into the pharmacology and clinical applications of these alkaloids has been performed in China during the past decade. The sophora alkaloids appear to inhibit viral replication, reduce destruction of liver cells, inhibit liver fibrosis and promote the flow of bile. Most of the clinical trials have been performed on HBV using oxymatrine extracted from *S. flavescens* and *S. subprostata*. Oxymatrine has been shown to be effective in normalizing ALT levels and clearing the HBV virus. The clinical effectiveness of oxymatrine for patients with hepatitis C has also been reported to show a reduction of viral load and inhibition of liver fibrosis, which appears to be a separate additional function of sophora alkaloids beyond inhibiting viral activity (*52-56*). Matrine was shown to reduce the formation of liver fibrosis that was caused by chemical damage to the liver (*57-58*). However, further research is needed to elucidate the effectiveness of these natural products for the treatment of chronic HCV, including their preparation and standardization.

The HCV genome possesses a unique open reading frame (ORF), encoding for a single long polyprotein that is processed by both host cellular peptidases and virus-encoded proteases (PR). HCV-PR is a primary and rational target in the development of anti-HCV agents. The medicinal herbs *Acacia nilotica, Boswellia carterii, Embelia schimperi, Piper cubeba, Quercus infectoria, Trachyspermum ammi* and *Syzygium aromaticum* extracts were investigated *in vitro* and showed significant inhibitory activity against HCV protease (*59*). In addition, the use of the botanical components glycyrrhizin, catechin, silymarin and phytosterols, and the antioxidants *N*-acetylcysteine were investigated for their efficacy in treating chronic hepatitis and affecting liver damage (*60*).

Herpes viruses and antiviral agents

Herpes viruses are common human pathogenic viruses, which include at least eight unique pathogenic strains, the neurotropic herpes simplex virus 1 (HSV-1) and

herpes simplex virus 2 (HSV-2), varicella zoster virus (VZV) (HSV-3), and lymphotropic human cytomegarovirus (HCMV) (HSV-4), EBV (HSV-5), HHV-6, HHV-7, and HHV-8. HSV-1 usually causes orolabial disease, and HSV-2 is associated more frequently with genital and newborn infections. HSV causes mild and self-limited disease of the mouth and lips or genitals. However, this disease can sometimes be life-threatening. Such is the case with neonatal HSV infection and HSV infections of the central nervous system. Furthermore, in the immunocompromised host, severe infection has been encountered and is a source of morbidity. Both viruses establish latent infections in sensory neurons and recurrent lesions at or near the point of entry into the body. Among HSV-related pathologies, genital herpes is an important sexually transmitted disease (STD) commonly caused by HSV-2. HSV-1 infections are very common and mostly affect adult people (*57*). In addition, HSV-2 infection may be a risk factor for the transmission of HIV (*61-62*).

Effective anti-herpes drugs, such as acyclovir, ganciclovir, valaciclovir, penciclovir, famciclovir, and vidarabine, are available for treatment. Acyclovir is the most commonly used drug for the treatment of HSV infection. However, a serious problem with the use of acyclovir is the emergence of drug resistance in treated patients. Drug-resistant strains of HSV frequently develop following therapeutic treatment. Resistance to acyclovir and related nucleoside analogues can occur following mutation in either HSV thymidine kinase (TK) or DNA polymerase. Virus strains associated with clinical resistance are almost always defective in TK production (*63*). Therefore, new antiviral agents exhibiting different mechanisms of action are urgently needed.

A large number of natural compounds from medicinal plant extracts, such as phenolics, polyphenols, terpenes (*e.g.*, mono-, di-, tri-), flavonoids, sugar-containing compounds, have been found to be promising anti-herpetic agents (*64*). Different kinds of anthraquinones from extracts of *Rheum officinale*, *Aloe barbadensis* (Aloe vera), *Rhamnus frangula, Rhamnus purshianus*, and *Cassia angustifolia* have been found to be quite active against HSV-1. Furthermore, inactivation of HSV-1 and HSV-2 and prevention of cell-to-cell virus spread by *Santolina insularis* essential oil has been found (*65*). Recently, 18 plants with ethnomedical backgrounds from different families were screened for antiviral activity against HSV-1, and three extracts, from *Hypericum mysorense, Hypericum hookerianum* and *Usnea complanta*, exhibited significant anti-HSV-1 activity at concentrations without toxic effects on cells *in vitro* (*66*). Furthermore, the study evaluated extracts of 23 medicinal species widely used in the traditional medicine of Nepal for the treatment of infectious and other diseases on their *in vitro* antiviral activity against influenza virus and herpes simplex-virus (HSV). Two species, *Bergenia ligulata* and *Nerium indicum*, showed the highest anti-influenza activity. *Holoptelia integrifolia* and *Nerium indicum* exhibited considerable antiviral activity against the herpes simplex virus. None of these extracts have shown any cytotoxic effects (*67*).

Development of screening systems for antiviral agents

Although the development of new anti-viral drugs for the treatment of many viral infected diseases is urgent, some viruses are not even established to propagate *in vitro*. The development of an effective screening system is crucial for the discovery of new antiviral drugs. However, safety concerns regarding using dangerous viruses have limited many laboratories to screen the antiviral activity of compounds, despite having the resources of active compounds either chemically synthesized or extracted from natural resources. We herein describe one example of the development of a new screening system for HIV-1 protease inhibitors, which was recently established in our laboratory. This system uses a cell line in which HIV-1 PR is expressed in a chimeric protein with the green fluorescent protein (GFP) by the tet-off system (*68*). This system can measure HIV-1 PR activity as a function of either the fluorescence of GFP or the cytotoxic activity of HIV-1 PR, which suppresses cell attachment when re-plated to a culture dish after the induction of HIV-1 PR expression (Figure 2). The system is virus-free, but the sensitivity is compatible with that of a system which uses a live virus for infection.

Perspective

Based on the specific assay system or screening approach, a large number of structurally unique antiviral compounds from medicinal plants (herbs) have been identified. The advantages of natural compounds are fewer side effects in comparison to orthodox medical drugs, and the production of synergistic effects for a more positive treatment outcome. However, the potential beneficial effects of these natural compounds need to be confirmed in large, rigorous trials. The continued discovery and development of new formulations of herbal medicines, containing a combination of multiple ingredients that synergistically act to potently and selectively inhibit virus replication at different stages and strengthen the impaired immune system, should be a potential therapeutic option in the future.

These natural lead structures can be chemically modified and improved through knowledge of the structure-activity relationship, mechanism of action, drug metabolism, molecular modeling and combinatorial chemistry studies. For the antiviral activity of these compounds, it is important to identify

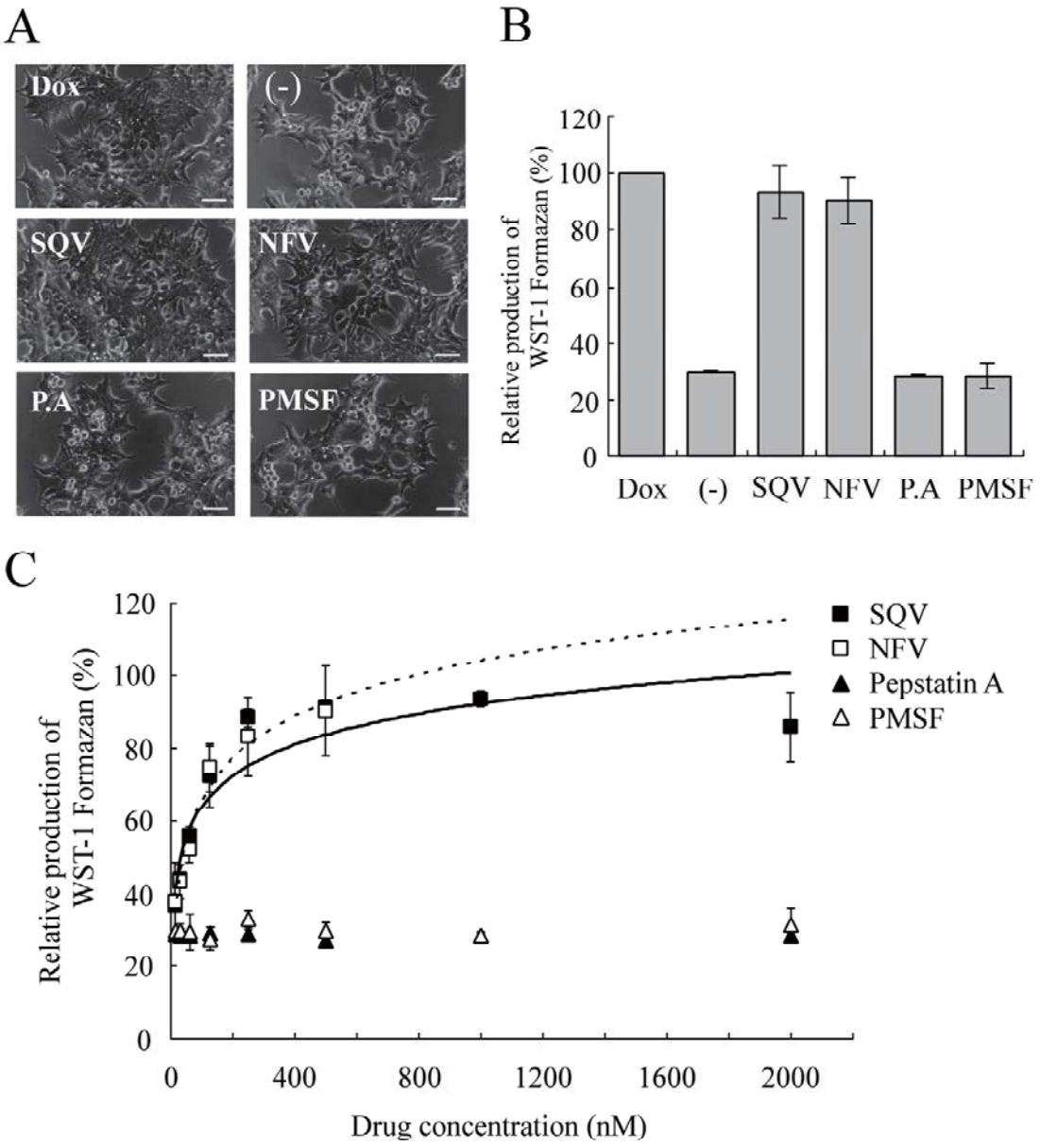

Figure 2. Development of a highly sensitive screening method for HIV-1 PR inhibitors. (A), Morphological change in the E-PR293. The E-PR293 cells were treated with 100 ng/mL of Dox or 1 mM of PR inhibitors, SQV, NFV, pepstain A (P.A) or PMSF. The scale bar indicates 50 mm; (B), The growth Figure 1. The HIV-1 replication life cycle. The replication life cycle of human immunodeficiency virus (HIV) have several specific steps (1-10), many of which are targets for antiviral drugs (A-E). of cells monitored by a WST-1 assay. The relative amount of WST-1 formazan to Dox-treated cells was calculated; (C), The dose-dependent activity of specific HIV-1 PR inhibitors on the cytotoxicty-based system. The cells were re-plated and then treated with SQV (closed square), NFV (opened square), pepstatin A (closed triangle), and PMSF (opened triangle) at various concentrations as indicated in the figure. The data represent the mean ± SD percentage.

virus specific targets and, when possible, to determine if this substance selectively interacts with the target. The assessment of the cytotoxicity of an antiviral compound is clearly an important part of the evaluation of a potential chemotherapeutic agent, since it should show neither acute nor long-term toxicity against the host. Determining the efficacy and toxicity of these agents as well as performing clinical trials together is expected to contribute to the generation of new drugs from such natural products.

Moreover, drug discovery must be improved greatly by new technologies emerging in the post-genomic era. Gene expression profile studies employing microarray technology could help to identify molecular targets of the biological activity of antiviral agents from natural products. On the other hand, the pathways for new drug discovery are broadened by the continuous improvement of technological platforms, including computer-aided drug design, high throughput screening, biochip, transgenic and RNAi technology. The continued investigation of active formulations, bioactive fractions, and isolated compounds is thus critical for continuing drug development in the 21st century.

References

1. Richman DD. Antiviral drug resistance. Antiviral Res

2006;71:117-121.
2. Yin PD, Das D, Mitsuya H. Overcoming HIV drug resistance through rational drug design based on molecular, biochemical, and structural profiles of HIV resistance. Cell Mol Life Sci 2006;63:1706-1724.
3. Shafer RW, Rhee SY, Pillay D, Miller V, Sandstrom P, Schapiro JM, Kuritzkes DR, Bennett D. HIV-1 protease and reverse transcriptase mutations for drug resistance surveillance. AIDS 2007;21:215-223.
4. De Clercq E. Strategies in the design of antiviral drugs. Nat Rev Drug Discov 2002;1:13-25.
5. Cragg GM, Newman DJ, Snader KM. Natural products in drug discovery and development. J Nat Prod 1997;60:52-60.
6. Harvey A. Strategies for discovering drugs from previously unexplored natural products. Drug Discov Today 2000;5:294-300.
7. Koehn FE, Carter GT. The evolving role of natural products in drug discovery. Nat Rev Drug Discov 2005;4:206-220.
8. Newman DJ, Cragg GM. Natural products as sources of new drugs over the last 25 years. J Nat Prod 2007;70:461-477
9. Jassim SAA, Naji MA. Novel antiviral agents: a medicinal plant perspective. J Applied Microbiol 2003;95:412-427.
10. UNAIDS/WHO "AIDS Epidemic Update: December 2006", http://data.unaids.org/pub/EpiReport/2006/2006_EpiUpdate_en.pdf
11. Simon V, Ho DD, Abdool Karim Q. HIV/AIDS epidemiology, pathogenesis, prevention, and treatment. Lancet 2006;368:489-504.
12. Opar A. New HIV drug classes on the horizon. Nat Rev Drug Discov 2007;6:258-259.
13. De Clercq E. Current lead natural products for the chemotherapy of human immunodeficiency virus (HIV) infection. Med Res Rev 2000;20:323-349.
14. El Safadi Y, Vivet-Boudou V, Marquet R. HIV-1 reverse transcriptase inhibitors. Appl Microbiol Biotechnol 2007;75:723-737.
15. Camarasa MJ, Velázquez S, San-Félix A, Pérez-Pérez MJ, Gago F. Dimerization inhibitors of HIV-1 reverse transcriptase, protease and integrase: a single mode of inhibition for the three HIV enzymes? Antiviral Res 2006;71:260-267.
16. Nair V, Chi G. HIV integrase inhibitors as therapeutic agents in AIDS. Rev Med Viol (in press).
17. Pommier Y, Johnson AA, Marchand C. Integrase inhibitors to treat HIV/AIDS. Nat Rev Drug Discov 2005;4:236-248.
18. Grinsztejn B, Nguyen BY, Katlama C, Gatell JM, Lazzarin A, Vittecoq D, Gonzalez CJ, Chen J, Harvey CM, Isaacs RD. Safety and efficacy of the HIV-1 integrase inhibitor raltegravir (MK-0518) in treatment-experienced patients with multidrug-resistant virus: a phase II randomized controlled trial. Lancet 2007;369:1261-1269.
19. Min BS, Hattori M, Lee HK, Kim YH. Inhibitory constituents against HIV-1 protease from Agastache rugosa. Arch Pharm Res 1999;22:75-77.
20. Min BS, Jung HJ, Lee JS, Kim YH, Bok SH, Ma CM, Nakamura N, Hattori M, Bae K. Inhibitory effect of triterpenes from Crataegus pinatifida on HIV-I protease. Planta Med 1999;65:374-375.
21. Rukachaisirikul V, Pailee P, Hiranrat A, Tuchinda P, Yoosook C, Kasisit J, Taylor WC, Reutrakul V. Anti-HIV-1 protostane triterpenes and digeranylbenzophenone from trunk bark and stems of Garcinia speciosa. Planta Med 2003;69:1141-1146.
22. Zhang HJ, Tan GT, Hoang VD, Hung NV, Cuong NM, Soejarto DD, Pezzuto JM, Fong HH. Natural anti-HIV agents. Part IV. Anti-HIV constituents from Vatica cinerea. J Nat Prod 2003;66:263-268.
23. Kashiwada Y, Hashimoto F, Cosentino LM, Chen CH, Garrett PE, Lee KH. Betulinic acid and dihydrobetulinic acid derivatives as potent anti-HIV agents. J Med Chem 1996;39:1016-1017.
24. Li F, Goila-Gaur R, Salzwedel K, Kilgore NR, Reddick M, Matallana C, Castillo A, Zoumplis D, Martin DE, Orenstein JM, Allaway GP, Freed EO, Wild CT. PA-457: a potent HIV inhibitor that disrupts core condensation by targeting a late step in Gag processing. Proc Natl Acad Sci U S A 2003;100:13555-13560.
25. Yu D, Wild CT, Martin DE, Morris-Natschke SL, Chen CH, Allaway GP, Lee KH. The discovery of a class of novel HIV-1 maturation inhibitors and their potential in the therapy of HIV. Expert Opin Investig Drugs 2005;14:681-693.
26. Yu D, Morris-Natschke SL, Lee KH. New developments in natural products-based anti-AIDS research. Med Res Rev 2007;27:108-132.
27. Ito M, Sato A, Hirabayashi K, Tanabe F, Shigeta S, Baba M, De Clercq E, Nakashima H, Yamamoto N. Mechanism of inhibitory effect of glycyrrhizin on replication of human immunodeficiency virus (HIV). Antiviral Res 1988;10:289-298.
28. Kitamura K, Honda M, Yoshizaki H, Yamamoto S, Nakane H, Fukushima M, Ono K, Tokunaga T. Baicalin, an inhibitor of HIV-1 production *in vitro*. Antiviral Res 1998;37:131-140.
29. Li BQ, Fu T, Dongyan Y, Mikovits JA, Ruscetti FW, Wang JM. Flavonoid baicalin inhibits HIV-1 infection at the level of viral entry. Biochem Biophys Res Commun 2000;276:534-538.
30. Min BS, Lee HK, Lee SM, Kim YH, Bae KH, Otake T, Nakamura N, Hattori M. Anti-human immunodeficiency virus-type 1 activity of constituents from Juglans mandshurica. Arch Pharm Res 2002;25:441-445.
31. Fassina G, Buffa A, Benelli R, Varnier OE, Noonan DM, Albini A. Polyphenolic antioxidant (-)-epigallocatechin-3-gallate from green tea as a candidate anti-HIV agent. AIDS 2002;16:939-941.
32. Lee JS, Kim HJ, Lee YS. A new anti-HIV flavonoid glucuronide from Chrysanthemum morifolium. Planta Med 2003;69:859-861.
33. Creagh T, Ruckle JL, Tolbert DT, Giltner J, Eiznhamer DA, Dutta B, Flavin MT, Xu ZQ. Safety and pharmacokinetics of single doses of (+)-calanolide a, a novel, naturally occurring nonnucleoside reverse transcriptase inhibitor, in healthy, human immunodeficiency virus-negative human subjects. Antimicrob Agents Chemother 2001;45:1379-1386.
34. Asres K, Seyoum A, Veeresham C, Bucar F, Gibbons S. Naturally derived anti-HIV agents. Phytother Res 2005;19:557-581.
35. Kostova I. Coumarins as inhibitors of HIV reverse transcriptase. Curr HIV Res 2006;4:347-363.
36. De Clercq E. Antiviral agents active against influenza A viruses. Nat Rev Drug Discov 2006;5:1015-1025.
37. Guan Y, Chen H. Resistance to anti-influenza agents. Lancet 2005;366:1139-1140.
38. Serkedjieva J, Toshkova R, Antonova-Nikolova S, Stefanova T, Teodosieva A, Ivanova I. Effect of a plant polyphenol-rich extract on the lung protease activities of influenza-virus-infected mice. Antivir Chem Chemother 2007;18:75-82.
39. Wang X, Jia W, Zhao A, Wang X. Anti-influenza agents from plants and traditional Chinese medicine. Phytother Res 2006;20:335-341.
40. Sokmen M, Angelova M, Krumova E, Pashova S, Ivancheva S, Sokmen A, Serkedjieva J. *In vitro* antioxidant activity of polyphenol extracts with antiviral

properties from *Geranium sanguineum* L. Life Sci 2005;76:2981-2993.
41. Miki K, Nagai T, Suzuki K, Tsujimura R, Koyama K, Kinoshita K, Furuhata K, Yamada H, Takahashi K. Anti-influenza virus activity of biflavonoids. Bioorg Med Chem Lett 2007;17:772-775.
42. Nagai T, Miyaichi Y, Tomimori T, Suzuki Y, Yamada H. *In vivo* anti-influenza virus activity of plant flavonoids possessing inhibitory activity for influenza virus sialidase. Antiviral Res 1992;19:207-217.
43. Serkedjieva J, Velcheva M. *In vitro* anti-influenza virus activity of the pavine alkaloid (-)-thalimonine isolated from Thalictrum simplex L. Antivir Chem Chemother 2003;14:75-80.
44. Rajbhandari M, Wegner U, Jülich M, Schöpke T, Mentel R. Screening of Nepalese medicinal plants for antiviral activity. J Ethnopharmacol 2001;74:251-255.
45. Kernan MR, Sendl A, Chen JL, Jolad SD, Blanc P, Murphy JT, Stoddart CA, Nanakorn W, Balick MJ, Rozhon EJ. Two new lignans with activity against influenza virus from the medicinal plant Rhinacanthus nasutus. J Nat Prod 1997;60:635-637.
46. Lai CL, Ratziu V, Yuen MF, Poynard T. Viral hepatitis B. Lancet 2003;362:2089-2094.
47. Lavanchy D. Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. J Viral Hepat 2004;11:97-107.
48. Patrick L. Hepatitis C: epidemiology and review of complementary/alternative medicine treatments. Altern Med Rev 1999;4:220-238.
49. Lauer G, Walker BD. Hepatitis C virus infection. N Engl J Med 2001;345:41-52.
50. Koziel MJ, Peters MG. Viral Hepatitis in HIV infection. N Engl J Med 2007;356:1445-1454.
51. Van Rossum TG, Vulto AG, De Man RA, Brouwer JT, Schalm SW. Review article: glycyrrhizin as a potential treatment for chronic hepatitis C. Aliment Pharmacol Ther 1998;12:199-205.
52. Liu J, Zhu M, Shi R, Yang M. Radix Sophorae flavescentis for chronic hepatitis B: a systematic review of randomized trials. Am J Chin Med 2003;31:337-354.
53. Lu LG, Zeng MD, Mao YM, *et al*. Oxymatrine therapy for chronic hepatitis B: a randomized double-blind and placebocontrolled multi-center trial. World J Gastroenterol 2003;9:2480-2483.
54. Lu LG, Zeng MD, Mao YM, Fang JY, Song YL, Shen ZH, Cao AP. Inhibitory effect of oxymatrine on serum hepatitis B virus DNA in HBV transgenic mice. World J Gastroenterol 2004;10:1176-1179.
55. Yu YY, Wang QH, Zhu LM, Zhang QB, Xu DZ, Guo YB, Wang CQ, Guo SH, Zhou XQ, Zhang LX. A clinical research on oxymatrine for the treatment of chronic hepatitis B. Zhonghua Gan Zang Bing Za Zhi 2002;10:280-281.
56. Mao YM, Zeng MD, Lu LG, *et al*. Capsule oxymatrine in treatment of hepatic fibrosis due to chronic viral hepatitis: a randomized, double blind, placebo-controlled, multicenter clinical study. World J Gastroenterol 2004;10:3269-3273.
57. Liu J, Manheimer E, Tsutani K, Gluud C. Medicinal herbs for hepatitis C virus infection: a Cochrane hepatobiliary systematic review of randomized trials. Am J Gastroenterol 2003;98:538-544.
58. Azzam HS, Goertz C, Fritts M, Jonas WB. Natural products and chronic hepatitis C virus. Liver Int 2007;27:17-25.
59. Hussein G, Miyashiro H, Nakamura N, Hattori M, Kakiuchi N, Shimotohno K. Inhibitory effects of Sudanese medicinal plant extracts on hepatitis C virus (HCV) protease. Phytother Res 2000;14:510-516.
60. Patrick L. Hepatitis C: epidemiology and review of complementary/alternative medicine treatments. Altern Med Rev 1999;4:220-238.
61. Whitley RJ, Roizman B. Herpes simplex virus infections. Lancet 2001;357:1513-1518.
62. Lingappa JR, Celum C. Clinical and therapeutic issues for herpes simplex virus-2 and HIV co-infection. Drugs 2007;67:155-174.
63. Coen DM, Schaffer PA. Antiherpesvirus drugs: a promising spectrum of new drugs and drug targets. Nat Rev Drug Discov 2003;2:278-288.
64. Khan MT, Ather A, Thompson KD, Gambari R. Extracts and molecules from medicinal plants against herpes simplex viruses. Antiviral Res 2005;67:107-119.
65. De Logu A, Loy G, Pellerano ML, Bonsignore L, Schivo ML. Inactivation of HSV-1 and HSV-2 and prevention of cell-to-cell virus spread by Santolina insularis essential oil. Antiviral Res 2000;48:177-185.
66. Vijayan P, Raghu C, Ashok G, Dhanaraj SA, Suresh B. Antiviral activity of medicinal plants of Nilgiris. Indian J Med Res 2004;120:24-29.
67. Rajbhandari M, Wegner U, Julich M, Schopke T, Mentel R. Screening of Nepalese medicinal plants for antiviral activity. J Ethnopharmacol 2001;74:251-255.
68. Fuse T, Watanabe K, Kitazato K, Kobayashi N. Establishment of a new cell line inducibly expressing HIV-1 protease for performing safe and highly sensitive screening of HIV protease inhibitors. Microbes Infect 2006;8:1783-1789.