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WHAT CLINICIANS NEED TO KNOW ABOUT ANTIVIRAL DRUGS AND VIRAL RESISTANCE

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During the last decade, significant advances have been made in the development and use of antiviral agents for the successful treatment of a number of viral infections.^{8, 51, 57} An expanding array of antiviral drugs are currently available for the management of infections caused by herpes simplex virus types 1 and 2 (HSV-1 and HSV-2), cytomegalovirus (CMV), varicella-zoster virus (VZV), influenza A virus, respiratory syncytial virus (RSV), human immunodeficiency virus type 1 (HIV-1), papillomaviruses, and hepatitis B and C viruses. The increased number and use of antiviral agents, however, has led to the emergence of drug-resistant viruses, particularly in immunocompromised patients such as those with acquired immunodeficiency syndrome (AIDS) or hematologic malignancy or those who have undergone organ transplantation. For comprehensive reviews on specific viruses, see references 26, 41, 64, 81-83, and 102.

Clinical situations that favor the development of resistance include long-term suppressive therapy, recurrent intermittent therapy, and the use of less than optimum doses of an antiviral agent. Generally, the emergence and isolation of drug-resistant viruses is associated more so with the therapeutic use of antiviral agents and does not seem to be caused by prophylactic treatment. As more patients fail to respond to appropriate therapy and additional antiviral agents are produced, it will also become important for diagnostic virology laboratories to provide

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rapid and practical antiviral susceptibility testing to assist physicians in defining drug resistance and choosing appropriate alternative therapies.

This review describes the major antiviral agents, their mechanisms of action, and the development of drug resistance following antiviral therapy. A brief overview of the available phenotypic and genotypic susceptibility assays for detecting antiviral resistance is also discussed.

MECHANISMS OF ANTIVIRAL DRUG ACTION AND VIRAL RESISTANCE

Herpesviruses

Human herpesviruses have a worldwide distribution and are a frequent cause of infections in immunocompetent and immunocompromised hosts. The number of severe and life-threatening conditions caused by these viruses is significant and includes localized oral, genital, and dermal infections, chickenpox, infectious mononucleosis, encephalitis, post-transplant lymphoproliferative disorder, and roseola infantum. There are a number of antiviral agents licensed for clinical use for the treatment and prophylaxis of herpesvirus infections (Table 1). Most are nucleoside analogs that require conversion to mono-, di-, and triphosphate forms by either cellular kinases, viral kinases, or both to selectively inhibit the replication of herpesviruses. This class includes vidarabine, acyclovir, valacyclovir, famciclovir, ganciclovir, cidofovir, idoxuridine, and trifluridine. A second class of antiviral drugs with action against herpesviruses is represented by the recently developed pyrophosphate analog foscarnet.

Vidarabine

Vidarabine (9- β -darabinofuranosyladenine; adenine arabinoside; araA; Vira-A) is an adenosine nucleoside analog with *in vitro* activity against HSV-1 and HSV-2, VZV, CMV, EBV, vaccinia virus, and hepatitis B virus and *in vivo* activity limited to HSV-1, HSV-2, and VZV.^{8, 51, 57} The drug is licensed for ophthalmic and intravenous use in the United States. Although the mechanism of action for vidarabine is unclear, the drug is phosphorylated by cellular kinases to its triphosphate form and prevents viral DNA synthesis through inhibition of the viral DNA polymerase. Vidarabine has efficacy for the treatment of herpes encephalitis, neonatal HSV infection, herpes keratitis, and mucocutaneous HSV infection and zoster in the immunocompromised host. The drug is not easy to use, however, because of its toxicity, poor solubility in water, and rapid deamination *in vivo* to a less active compound, arahypoxanthine. As such, vidarabine is seldom used and has been largely replaced by acyclovir. Resistance to vidarabine has not been identified in a clinical setting, although drug-resistant isolates can be induced in the laboratory and are caused by an altered viral DNA polymerase.²² Vidarabine has

Table 1. AVAILABLE ANTIVIRAL AGENTS FOR HERPESVIRUSES: CLINICAL INDICATIONS AND MECHANISMS OF RESISTANCE

Antiviral Agent	Approved Clinical Indications	Resistance Mechanisms
Nucleoside Analog		
Acyclovir	Primary and recurrent herpes, HSV encephalitis, neonatal herpes, treatment and prophylaxis of mucocutaneous HSV infection, primary varicella or zoster in immunocompromised host	Altered or deficient viral thymidine kinase; altered DNA polymerase
Cidofovir	CMV retinitis in patients with AIDS	Altered DNA polymerase
Famciclovir	Recurrent genital HSV and zoster infections in adults	Altered or deficient viral thymidine kinase; altered DNA polymerase
Ganciclovir	Treatment of CMV in immunocompromised host, prophylaxis of transplant and HIV-infected patients	Diminished drug phosphorylation; altered DNA polymerase
Idoxuridine	Herpes keratitis (topical use only)	Unknown
Trifluridine	Herpes keratitis (topical use only)	Unknown
Valacyclovir	Recurrent genital HSV and zoster infections in immunocompetent adults	Altered or deficient viral thymidine kinase; altered DNA polymerase
Vidarabine	HSV encephalitis, neonatal herpes, herpes keratitis, zoster in immunocompromised host	Altered DNA polymerase
Pyrophosphate Analog		
Foscarnet	Mucocutaneous acyclovir-resistant HSV infections, CMV retinitis	Altered DNA polymerase

HSV = herpes simplex virus; CMV = cytomegalovirus.

in vitro activity against acyclovir-resistant HSV and VZV isolates, but the drug has not proven useful for the treatment of these isolates in HIV-infected patients.⁸⁹

Acyclovir

Acyclovir (9-[2-hydroxyethoxymethyl] guanine; acycloguanosine; Zovirax) is a guanosine analog that has good in vitro activity against HSV-1, HSV-2, and VZV but limited activity against CMV, EBV, and human herpesvirus-6 (HHV-6).¹¹⁶ It is most useful clinically for the treatment of HSV and VZV infections. Acyclovir accumulates only in

cells infected with herpesviruses in which it is phosphorylated by a viral-specific thymidine kinase to its monophosphate form. It is further phosphorylated to the diphosphate and active triphosphate forms by cellular enzymes; the acyclovir triphosphate inhibits viral DNA polymerase by acting as an immediate DNA chain terminator. Acyclovir is efficacious for the treatment of primary and recurrent HSV and VZV infections, HSV encephalitis, neonatal herpes, and the management of mucocutaneous HSV infection. Resistance to acyclovir can result from mutations in the genes encoding the viral thymidine kinase (TK) or the viral DNA polymerase.^{23, 42, 53, 94} Mutations in the gene for TK can result in viral isolates that are either deficient (TK⁻) in or have altered production (TK^A) of viral thymidine kinase activity.^{23, 34, 42, 50, 94} Viral isolates with a TK⁻ phenotype cause a decrease or lack of acyclovir phosphorylation, whereas TK^A strains result in altered substrate binding properties for acyclovir.

Valacyclovir

Valacyclovir (L-valine, 2-[(2-amino-1,6-dihydro-6-oxo-9H-purin-9-yl) methoxy] ethyl ester; Valtrex) is the L-valyl ester of acyclovir that has been recently licensed for treatment of recurrent genital HSV infections and herpes zoster in immunocompetent adults.^{1, 11} The drug is rapidly and extensively converted to acyclovir after oral administration, resulting in a 3 to 5 fold increase in the bioavailability of acyclovir over that observed for oral doses of acyclovir itself. To date, clinical resistance to valacyclovir and comparative susceptibilities of acyclovir-resistant clinical isolates to valacyclovir and acyclovir have not been reported. The expected mechanisms of viral resistance to valacyclovir, however, are likely to be the same as those for acyclovir.

Famciclovir

Famciclovir (2-[2-(2-amino-9H-purin-9-yl)ethyl]-1,3-propanediol diacetate; Famvir) is a synthetic guanine derivative that is the orally administered prodrug of the antiviral compound penciclovir.^{17, 113} It has been approved for use in adults for the treatment of recurrent genital HSV and herpes zoster. It is rapidly and extensively absorbed following oral administration and is converted to its active triphosphate form of penciclovir by mechanisms that are similar to acyclovir. Like acyclovir, penciclovir has good in vitro activity against HSV and VZV and limited activity against EBV and CMV. Unlike acyclovir, penciclovir is not an obligate chain terminator but is an effective inhibitor of DNA chain elongation. Also, despite a less powerful inhibition of the viral DNA polymerase than acyclovir, famciclovir has a much higher oral bioavailability, and its active form is phosphorylated much more efficiently and has a significantly longer intracellular half-life in herpes-infected cells. This may ensure prolonged antiviral activity in virus-infected cells and may be responsible for the lower and more convenient dosage regimen

of famciclovir. Resistance to the active metabolite of famciclovir has been documented, but the mechanisms have not been fully elucidated. Acyclovir-resistant clinical isolates of HSV and VZV that are deficient in viral thymidine kinase are cross-resistant to penciclovir⁹¹; penciclovir is active *in vitro*, however, against some acyclovir-resistant isolates of HSV and VZV with an altered thymidine kinase or DNA polymerase.^{17, 107} Foscarnet-resistant HSV isolates seem to be susceptible to penciclovir *in vitro*.^{17, 91}

Ganciclovir

Ganciclovir (9-[(1,3-dihydroxy-2-propoxy)methyl] guanine); DHPG; Cytovene) is another nucleoside analog of guanine with antiviral activity against all of the herpesviruses.⁴⁰ It is unique, however, in that it is up to 100-fold more potent than acyclovir against CMV. Although the *in vitro* activity of ganciclovir is equal to acyclovir for the inhibition of HSV and VZV, its toxicity and lack of selectivity for viral enzymes has limited the clinical use of ganciclovir to the treatment and prophylaxis of serious CMV infections in immunocompromised patients. Ganciclovir is activated to its monophosphate form by a viral-specific thymidine kinase in HSV-infected cells and, in CMV-infected cells, by a phosphotransferase encoded by the UL97 gene of CMV.¹⁰⁵ Conversion to the active triphosphate form of ganciclovir is accomplished by cellular kinases. Ganciclovir triphosphate inhibits viral DNA synthesis by competitive inhibition of viral DNA polymerases and by direct incorporation into viral DNA, thereby terminating viral DNA elongation. The mechanisms of resistance to ganciclovir in CMV include alterations in the gene encoding the viral DNA polymerase^{5, 71, 108} and mutations in the catalytic domain of the phosphotransferase gene leading to a deficiency in drug phosphorylation.^{20, 21, 101, 117}

Cidofovir

Cidofovir ((S)-1-[3-hydroxy-2-(phosphonylmethoxy) propyl] cytosine; HPMP) is a novel monophosphate nucleotide analog that is a selective and potent inhibitor of CMV replication and has antiviral activity against other herpesviruses.⁶⁵ It is licensed in the United States as an alternative to ganciclovir or foscarnet for the treatment and prophylaxis of CMV retinitis in patients with AIDS. Because it contains a phosphonate group, viral-specific enzymes are not required for initial phosphorylation. Unlike ganciclovir, cidofovir is phosphorylated by cellular enzymes to its active diphosphate form, which selectively inhibits the viral DNA polymerase. This may prove advantageous for the alternative treatment of herpesvirus resistance to nucleoside analogs caused by deficient or altered viral enzymes that are required for conversion of certain drugs to their monophosphate form.^{60, 98} The active diphosphate form also has a long intracellular half-life, allowing for a more convenient dosage regimen than either ganciclovir or foscarnet. Ganciclovir

and foscarnet must be administered 2 or 3 times daily during initial treatment and once daily for maintenance, whereas cidofovir can be given once weekly for 2 weeks followed by once every other week. In vitro combinations of cidofovir with acyclovir, ganciclovir, foscarnet, and zidovudine have demonstrated additive or synergistic inhibition of several human CMV strains. Although viral resistance to cidofovir has been demonstrated in vitro, it has not been documented in patients treated with the drug.⁶⁵

Idoxuridine and Trifluridine

Idoxuridine (5-iodo-2'-deoxyuridine; IUdR) and trifluridine (5-trifluoromethyl-2'-deoxyuridine; trifluorothymidine; TFT; Viroptic) are two thymidine nucleoside analogs used for the topical treatment of ocular herpes simplex virus infections.^{8, 51, 57} These drugs represent some of the earliest antiviral compounds developed, and their use is limited because of toxicity and insufficient potency. Idoxuridine and trifluridine are phosphorylated to the monophosphate form by viral thymidine kinase in HSV-infected cells and to the triphosphate form by cellular enzymes. Disruption of viral replication is thought to be related to inhibition of the viral DNA polymerase, but the exact mechanism of action of these drugs is unknown. Currently, only trifluridine is licensed for use in the United States. It has activity against HSV-1, HSV-2, vaccinia virus, and some strains of adenovirus. Trifluridine is indicated for the topical treatment of primary keratoconjunctivitis and recurrent epithelial keratitis caused by HSV-1 and HSV-2 and has been used as a topical treatment for acyclovir-resistant chronic mucocutaneous genital HSV infections in HIV-infected patients. Viral resistance to idoxuridine and trifluridine has been demonstrated in vitro, but resistance following clinical use has not been reported. Resistant mutants are thought to possess deficient or altered thymidine kinase activity, although the mutants have not been extensively characterized.⁶² Isolates resistant to idoxuridine are cross-resistant to trifluridine.

Foscarnet

Foscarnet (trisodium phosphonoformate; Foscavir) is unlike the described nucleoside analogs for use against herpesviruses in that it is an organic analog of inorganic pyrophosphate.¹¹⁵ It has antiviral activity against HSV-1, HSV-2, CMV, VZV, EBV, HHV-6, hepatitis B virus, and HIV-1. Foscarnet exerts its inhibitory effect by acting at the pyrophosphate binding site on viral-specific DNA polymerases and reverse transcriptases. Phosphorylation by thymidine kinase or other kinases is not required for activation of foscarnet. Thus, acyclovir-resistant HSV isolates that are TK⁻ or TK^A mutants and CMV UL97 mutants resistant to ganciclovir may be sensitive to foscarnet. Resistance to foscarnet is caused by mutations in the viral DNA polymerase gene^{53, 108}, acyclovir- or ganciclovir-resistant isolates with altered DNA polymerase activity

may be resistant to the drug. Foscarnet is indicated for the treatment of ganciclovir-resistant CMV retinitis in patients with AIDS^{32, 55} and for the treatment of acyclovir-resistant mucocutaneous HSV infections in immunocompromised patients.^{19, 38, 87, 89, 92, 112, 118} Acyclovir-resistant VZV has also been successfully treated with foscarnet.^{88, 97}

Clinical Importance of Herpesvirus Resistance

Resistance of clinical HSV isolates to acyclovir is a problem of growing concern, occurring at rates of 2% to 14% in the immunocompromised host following prolonged courses of continuous or intermittent suppressive therapy.^{36, 114} Acyclovir-resistant strains of HSV have been recovered from patients with AIDS, bone marrow and solid organ transplant recipients, and patients receiving cancer chemotherapy.* Children with agammaglobulinemia or severe combined immunodeficiency and a neonate with laryngeal herpes have also been identified with acyclovir-resistant HSV infections.^{78, 95} In these patients, resistant HSV can be associated with chronic, progressive, debilitating disease that does not respond to antiviral treatment. Resistance to acyclovir, however, is usually not associated with more severe or prolonged HSV infection.^{75, 114} Also, acyclovir-resistant isolates may become latent and cause recurrent disease.^{36, 87} In some patients, recurrences are caused by drug-sensitive isolates after the successful treatment of infection with a resistant virus.^{24, 62} Most acyclovir-resistant clinical isolates of HSV are TK⁻ mutants, although TK^Δ and DNA polymerase mutants have also been reported.

The recovery of acyclovir-resistant clinical isolates of HSV from immunocompetent patients is rare.^{36, 66, 77} Acyclovir-resistant HSV isolates have been obtained from individuals before therapy⁸⁰ and from otherwise healthy patients on chronic suppressive therapy for genital herpes,^{43, 103} although long-term treatment does not seem to increase the risk for HSV resistance nor has a correlation been established between these isolates and the clinical outcome in immunocompetent hosts. A single report suggests recurrent acyclovir-resistant HSV infection in an immunocompetent individual that correlated with clinical failure of antiviral therapy.⁵⁹ This virus possessed a TK^Δ phenotype and may have been acquired from an HIV-infected sexual contact who received long-term acyclovir therapy for recurrent genital herpes. Foscarnet has been employed successfully as an alternative antiviral agent for treating TK⁻ or TK^Δ acyclovir-resistant HSV infections.^{19, 38, 87, 89, 92, 112, 118} However, *in vivo* resistance to this drug has also been documented.^{13, 86, 90, 91} Foscarnet-resistant HSV isolates show absolute or borderline susceptibility to acyclovir, possibly indicating that acyclovir and foscarnet possess different binding sites on the viral DNA polymerase.⁹¹ High-dose oral or intravenous acyclovir, either alone or in combination with foscarnet, has been employed for the successful treatment of foscarnet-resistant HSV isolates.³⁵ Alternative drugs, such as famciclovir, valacyclovir, cidofovir,

*References 6, 7, 12, 13, 25, 39, 45, 70, 76, 86, and 92.

and trifluridine, also may be considered for the treatment of foscarnet-resistant HSV strains,^{60, 98} although their use in treating such isolates requires further investigation. Clinical isolates resistant to both acyclovir and foscarnet have also been reported.⁹¹

Why immunocompromised patients are at greater risk of acquiring drug-resistant HSV strains compared to hosts with normal immunity is still unclear. It has been suggested that an increased viral burden during infection of immunocompromised patients favors a larger gene pool for the emergence of viral mutations during the selective pressure of antiviral therapy.³⁶ Also, clinical isolates of HSV may represent a heterogeneous population containing both susceptible and resistant viruses⁸⁶; drug-resistant strains may be preferentially selected during antiviral therapy.

Acyclovir is the preferred drug for the treatment of primary VZV infection or herpes zoster in immunocompromised hosts. However, acyclovir-resistant clinical isolates of VZV have been recovered from both adult and pediatric AIDS patients who presented with indolent and disseminated, atypical hyperkeratotic or nodular lesions following long-term therapy.^{54, 68, 73, 79, 88, 99} Similar to acyclovir-resistant HSV isolates, most acyclovir-resistant clinical isolates of VZV have deficient or altered thymidine kinase activity.^{14, 16, 107} Several VZV isolates, however, have been reported to be resistant to both acyclovir and the alternative drug foscarnet.^{44, 79, 88} This is most likely caused by mutations in the viral DNA polymerase gene but may also be a result of combined mutations in the thymidine kinase and DNA polymerase genes.

Resistance of CMV to ganciclovir has been reported in immunocompromised patients,* particularly patients with AIDS receiving long-term ganciclovir treatment for CMV retinitis.^{31, 33, 37, 55, 109} Three distinct scenarios have been described for the development of ganciclovir-resistant CMV in some patients, including infection with a resistant virus before ganciclovir treatment, infection with a susceptible virus that became resistant over the course of therapy, and infection initially by a susceptible virus with the emergence of a genetically distinct resistant virus during treatment.³⁷ A prevalence rate of 8% has been reported for ganciclovir-resistant CMV among AIDS patients who received prolonged treatment with intravenous ganciclovir for CMV retinitis.³¹ Ganciclovir-resistant clinical isolates also have been recovered from bone marrow transplant recipients and a patient with chronic lymphocytic leukemia.^{32, 37, 58, 96} Although foscarnet is the alternative therapy for ganciclovir-resistant CMV infections, clinical CMV isolates resistant to foscarnet alone or in combination with ganciclovir resistance have been described.^{58, 67, 96, 104, 108} More recently, double resistance to ganciclovir and foscarnet has been reported for four clinical isolates of CMV obtained from four different AIDS patients following multiple sequential and combined courses of treatment with either or both drugs.⁹³ Each isolate represented a single CMV strain and ganciclovir resistance was

*References 4, 5, 15, 31-33, 37, 55, 58, 96, and 109.

caused by a mutation in the UL97 phosphotransferase gene, whereas mutations in the UL54 DNA polymerase gene were responsible for resistance to foscarnet.⁵

Human Immunodeficiency Virus

A number of antiretroviral drugs have been approved for the treatment of HIV infection,^{27, 69} and they are divided into three main classes based on their mode of inhibition of viral replication (Table 2). The first class represents the nucleoside analogs that inhibit the viral RNA-dependent DNA polymerase (reverse transcriptase) of HIV. Included in this drug class are zidovudine (3'-azido-3'-deoxythymidine; AZT; Retrovir), didanosine (2',3'-dideoxyinosine; ddI; Videx), zalcitabine (dideoxycytidine; ddC; Hivid), stavudine (2', 3'-didehydro-2', 3'-dideoxythymidine; d4T; Zerit), and lamivudine (3'-thia-2', 3'-dideoxycytidine; 3TC; Epivir). A second class of reverse transcriptase inhibitors are represented by the non-nucleoside analogs nevirapine (dipyridodiazepinone; Viramune) and delavirdine (bis (heteroaryl)-piperazine; Rescriptor). The third class are protease inhibitors and include saquinavir (RO-31-8959; Invirase), ritonavir (ABT-538; Norvir), indinavir (MK-639; Crixivan), and nelfinavir (AG-1343; Viracept). Therapy with these antiretroviral agents, either alone or in combinations, may be beneficial to prolong survival, to reduce the incidence and severity of opportunistic infections in patients with advanced HIV disease, and to delay disease progression in asymptomatic HIV-infected patients.

Nucleoside Analogs

The nucleoside analogs are all dideoxynucleosides that are converted by cellular enzymes to their mono-, di-, and active triphosphate forms to interact with the substrate binding site to competitively inhibit the activity of the viral reverse transcriptase.^{3, 27, 69} The triphosphate form can also act as an alternate substrate that is incorporated into the growing DNA chain, leading to chain termination. The spectrum of activity of these drugs extends to viruses that depend on reverse transcriptase or similar enzymes and includes HIV, other retroviruses, and hepatitis B virus.

Non-Nucleoside Analogs

The non-nucleoside analogs target the same enzyme as the nucleoside analogs, but they interact with the enzyme in a different way and their antiviral activity does not require any intracellular conversion of the drugs.^{27, 29, 69} These compounds interact directly with the reverse transcriptase, acting noncompetitively at an allosteric, nonsubstrate binding site of the enzyme. The non-nucleoside reverse transcriptase inhibitors are highly selective and potent inhibitors of HIV-1 replication

Table 2. AVAILABLE ANTIVIRAL AGENTS FOR HUMAN IMMUNODEFICIENCY VIRUS: CLINICAL INDICATIONS AND MECHANISMS OF RESISTANCE

Antiviral Agent	Approved Clinical Indications	Resistance Mechanisms
Nucleoside Analog		Altered viral reverse transcriptase
Zidovudine (AZT)	Treatment of HIV infection, prevention of maternal-fetal HIV transmission	
Didanosine (ddI)	Treatment of HIV infection, AZT intolerance or AZT treatment failure in HIV infection	
Zalcitabine (ddC)	Intolerance or treatment failure with AZT and ddI, used as combination therapy with other antiretroviral agents	
Stavudine (d4T)	Treatment of HIV infection after prolonged, prior AZT therapy	
Lamivudine (3TC)	Treatment of HIV infection in combination with AZT	
Non-Nucleoside Analog		Altered viral reverse transcriptase
Nevirapine	Treatment of HIV infection in combination with nucleoside analogs	
Delavirdine	Treatment of HIV infection in combination with nucleoside analogs	
Protease Inhibitors		Altered viral protease
Indinavir	Treatment of HIV infections as a monotherapy or in combination with nucleoside analogs	
Ritonavir	Treatment of HIV infections as a monotherapy or in combination with nucleoside analogs	
Saquinavir	Treatment of HIV infections in combination with nucleoside analogs	
Nelfinavir	Treatment of HIV infections in combination with nucleoside analogs	

but are inactive against HIV-2 or other retroviruses. They are equally effective against AZT-sensitive or AZT-resistant isolates. Similar to the nucleoside analogs, they also have excellent oral bioavailability.

Protease Inhibitors

The protease inhibitors are a newly developed class of antiretroviral drugs that selectively inhibit the viral proteases of both HIV-1 and HIV-

2.^{27, 69, 84} The HIV protease cleaves the viral gag-pol polyprotein precursor into the gag proteins and the enzymes integrase and protease during virus assembly and maturation. The enzyme is required for viral infectivity. Inhibition of HIV protease activity, therefore, leads to the development of immature, noninfectious viral particles. Protease inhibitors act at a postintegration step of HIV replication and have the distinct advantage over reverse transcriptase inhibitors of blocking late stages of viral replication and, thereby, preventing the spread of HIV from cells already infected with the virus. The major disadvantages of the protease inhibitors are their low solubility in aqueous medium and poor oral bioavailability. However, the oral bioavailability and solubility of certain protease inhibitors have been significantly enhanced by appropriate chemical modifications to the drugs.

Resistance to Antiretroviral Drugs

The development of HIV resistance to various antiretroviral drugs has been well documented in patients receiving antiretroviral therapy. The number of original articles on this subject is extensive and the readers should refer to references 26, 28, 29, 82-84, and 102 for more comprehensive reviews of the literature. The emergence of HIV antiviral drug resistance is thought to be a function of the length of therapy, stage of disease or viral burden, and the high spontaneous mutation rate in the viral genome during active replication of the virus. Prolonged therapy and advancing disease increase the likelihood of developing viral resistance. In vitro and in vivo resistance to various antiretroviral drugs is caused by the accumulation of specific mutations in the coding regions of either the reverse transcriptase or the protease of the virus, leading to single amino acid substitutions in the viral protein targets. The extent of resistance seems to be correlated with the number of mutations, although individual mutations can be interactive, causing changes in the resistance or susceptibility of HIV isolates.

Long-term monotherapy with nucleoside analogs such as AZT, ddI, ddC, and d4T have led to the recovery of drug-resistant HIV isolates.^{3, 28} Resistance seems to develop within 6 to 12 months of initial therapy for these drugs. Mutations in the reverse transcriptase gene at codons 41, 67, 70, 215, and 219 confer resistance to AZT. Individual mutations cause only low-level resistance, whereas four mutations are required for high-level resistance to AZT. High-level AZT resistance develops with time and has been associated with a more rapid clinical progression of disease while receiving therapy. Also, AZT-resistant virus has been transmitted by parenteral exposure to HIV-infected blood, sexual contact, and from mother to fetus. Similarly, mutations in the reverse transcriptase gene at codons 74 or 184 are associated with resistance to ddI and cross-resistance to ddC, whereas the change at codon 74 can also suppress the effect of the AZT resistance mutation at codon 215 and restore sensitivity of the isolate to AZT. Resistance to ddC also has been associated with mutations at codons 65, 69, 75, and 215, whereas cross-resistance to ddI

has been observed with mutations at codons 65 and 75. Additionally, point mutations at positions 50 and 75 are associated with resistance to d4T and those at positions 65 and 184 with resistance to 3TC. Under the selective pressure of AZT, cross-resistance to other antiretroviral agents is uncommon and confined only to nucleoside analogs with an azido group. Therefore, AZT-resistant viruses will remain susceptible to all other nucleoside analogs, the non-nucleoside analogs, and the protease inhibitors. Unlike the other nucleoside analogs, resistance to 3TC monotherapy develops within days or weeks because high levels of resistance are conferred by only a single mutation at codon 184. The same mutation at codon 184 also can reverse the effects of multiple AZT resistance mutations.

Rapid development of resistant virus is also observed in many patients treated with the non-nucleoside analogs.^{28, 29} Resistance to these drugs occurs within days to weeks and is the result of any one of a number of possible mutations in the gene for the reverse transcriptase. The mutations occur around the site in which the non-nucleoside analogs bind and, as is the case for nevirapine, cluster at codons 98, 100, 103, 106, 108, 181, 188, and 190. Resistance to one of the non-nucleoside analogs usually confers complete cross-resistance to all members of the group. The mutation at codon 181 causes high-level nevirapine resistance and cross-resistance to most other non-nucleoside reverse transcriptase inhibitors, while suppressing the effect of AZT resistance mutations. Cross-resistance to AZT and nevirapine can develop. A mutation at codon 236 has been described for the resistance of HIV isolates to delavirdine, which also confers an increased susceptibility of the isolates to other non-nucleoside inhibitors. Because of the rapid development of viral resistance with the non-nucleoside analogs, the potential of these agents as monotherapy is limited and they are only being used in combination with other antiretroviral drugs.

Human immunodeficiency virus isolates resistant to protease inhibitors can be selected by laboratory passage in the presence of a given drug and *in vivo*.⁸⁴ A number of mutations within the protease gene have been recognized that confer viral resistance to the protease inhibitors and include codons 8, 32, 46, 48, 82, 90, and 97. Cross-resistance to multiple protease inhibitors following resistance to a single protease inhibitor also has been described.

Combination Therapy for Treatment of HIV Infection

Because monotherapy with available antiretroviral agents has had limited success in the prolonged treatment of HIV infection and has led to the occurrence of drug-resistant viruses, the efficacy of combination therapy is now being studied.^{46, 61, 63} The ultimate goals of combination therapy are to more effectively inhibit viral replication and to reduce the viral burden to extremely low levels for as long as possible to preserve immune function, prevent the development of drug resistance, and interrupt progression of disease. With this in mind, certain combina-

tions of drugs may demonstrate additive or synergistic antiviral effects, such as that already being described in ongoing studies of nucleoside analogs with each other or with the non-nucleoside analogs or protease inhibitors. Also, the combined selection of antiretroviral drugs that act on different stages of viral replication and do not induce cross-resistance or create increased toxicity may be beneficial for the adequate management of HIV disease.

Respiratory Viruses

Respiratory viruses represent a heterogeneous group of agents that can infect the human respiratory tract and cause significant morbidity and mortality. Severe illness commonly occurs in infants, the elderly, the chronically ill, and the immunocompromised. The viruses that are a major cause of acute respiratory disease include influenza A and B viruses, respiratory syncytial virus, parainfluenza virus types 1, 2, and 3, adenoviruses, rhinoviruses, and respiratory coronaviruses. The treatment of infections with these viruses, however, is mostly supportive and the number of available antiviral drugs in use is limited. Oral amantadine or rimantadine has been employed for influenza A viral illness¹¹¹ and aerosolized ribavirin^{2, 52} has been used for RSV infections (see Table 3).

Amantadine and Rimantadine

Amantadine (1-adamantanamine hydrochloride; Symmetrel) and its alpha-methyl derivative rimantadine (Flumadine) are primary symmetrical amines that are inhibitory to the replication of influenza A virus from each of the three antigenic subtypes isolated from humans (i.e., H1N1, H2N2, H3N2); neither drug has sufficient activity against influenza B virus isolates.^{8, 111} Both drugs are licensed in the United States for the treatment and prophylaxis of influenza A virus, but rimantadine has fewer side effects and a longer half-life for easier once-a-day use. Administration of either drug within 48 hours after the onset of illness can reduce the severity and duration of influenza disease among healthy individuals. Their mechanisms of action are identical and involve interference with the function of the transmembrane domain of the viral M2 matrix protein, thereby inhibiting the uncoating of the virus. These drugs also have been shown to prevent virus assembly during later stages of viral replication. Amantadine-resistant and rimantadine-resistant influenza A isolates have been recovered from adult and pediatric patients undergoing therapy or prophylaxis during epidemics of influenza disease.^{9, 30, 47-49, 74} Resistance develops rapidly, occurring within 2 to 3 days of starting treatment. Viral resistance is caused by point mutations in the gene encoding the M2 protein¹⁰ and the resistant isolates are transmissible to close contacts and cause typical influenza illness.^{9, 47, 48} Cross-resistance between amantadine and rimantadine has been reported and is complete. The role of alternative antiviral com-

pounds for the prevention or therapy of influenza A infection has not been fully defined.

Ribavirin

Ribavirin (1- β -D-ribofuranosyl-1H-1,2,4-triazole-3-carboxamide; Virazole) is a synthetic nucleoside analog resembling guanosine and inosine. The drug has a broad in vitro antiviral activity against many RNA and DNA viruses, including RSV, influenza A and B viruses, parainfluenza viruses, adenoviruses, herpesviruses, bunyaviruses, arenaviruses, reoviruses, and HIV-1.^{2, 8, 52} It is converted by cellular enzymes to its mono-, di-, and triphosphate forms; the mono- and triphosphate forms are active. Although its exact mechanism of action is unknown, it seems to interfere with the expression of viral mRNA and to inhibit viral protein synthesis. Ribavirin has reported clinical efficacy against RSV, influenza A and B, hantavirus, and Lassa fever virus infections but is only licensed in the United States for the aerosolized treatment of hospitalized infants and young children with severe lower respiratory tract infections caused by RSV. The expense and mode of therapy limits the use of this antiviral drug. Viral resistance to ribavirin following patient therapy has not been documented to date.

Hepatitis B and C Viruses and Human Papillomaviruses

Interferon- α

Interferons belong to a class of immunomodulators called cytokines that are small glycoproteins or peptides that function like hormones to regulate the immune system. They are also released by eukaryotic cells in response to viral infection and act on surrounding uninfected cells to render them more resistant to infection by various DNA and RNA viruses.^{8, 57} Interferons bind to specific cell-surface receptors, are internalized by the cell, and induce cellular activities that prevent viral penetration or uncoating, synthesis, and expression of viral mRNA, viral protein synthesis, and viral assembly and release. There are three major classes of human interferons, including interferon- α , interferon- β , and interferon- γ . Only interferon- α has been approved for use in the treatment of certain viral infections (Table 3). Chronic liver disease caused by hepatitis B and C viruses and condyloma acuminatum caused by human papillomaviruses have been shown to respond to treatment with interferon- α . Also, interferon- α has antiretroviral activity against the replication of HIV by directly inhibiting virus assembly or release. The in vivo actions of interferon- α , its role as an antiviral agent, and how viruses avoid the effects of this drug is complex and is not yet completely understood.

Table 3. AVAILABLE ANTIVIRAL AGENTS FOR MISCELLANEOUS VIRUSES: CLINICAL INDICATIONS AND MECHANISMS OF RESISTANCE

Antiviral Agent	Approved Clinical Indications	Resistance Mechanisms
Primary Symmetrical Amine Amantadine or Rimantidine	Treatment or prophylaxis of influenza A virus	Altered transmembrane domain of M2 protein
Nucleoside Analog Ribavirin	Treatment of hospitalized infants and young children with severe respiratory syncytial virus infections	Unknown
Cytokine		
Interferon- α -n3	Intralesional treatment of refractory or recurring external condylomata acuminata	Unknown
Interferon- α -2a	Treatment of Kaposi's sarcoma, treatment of hairy cell leukemia	Unknown
Interferon- α -2b	Treatment of hepatitis B or C chronic liver disease, treatment of external condylomata acuminata, treatment of Kaposi's sarcoma, treatment of hairy cell leukemia	Unknown

ANTIVIRAL SUSCEPTIBILITY TESTING

Clinical Indications

The described emergence of antiviral drug resistance has led to a definite need for in vitro antiviral susceptibility testing. Laboratory confirmation of drug resistance may predict treatment failure, define cross-resistance to other antiviral agents, and lead to the institution of the most appropriate alternative therapy. Clinical situations that warrant the use of antiviral susceptibility testing include failure of HSV or VZV lesions to resolve or the appearance of new lesions while on acyclovir therapy, progressive CMV disease during ganciclovir therapy, continuous shedding or transmission of influenza A virus during treatment or prophylaxis with amantadine or rimantadine, and progression of HIV disease as measured by increased HIV-1 RNA plasma levels or decreased CD4⁺ cell counts in patients during antiretroviral therapy. To this end, both phenotypic and genotypic antiviral susceptibility assays have been developed for the determination of viral drug resistance.

Phenotypic Assays

A number of phenotypic assays have been described for testing the susceptibility of viruses to antiviral agents.^{72, 106} Generally, phenotypic antiviral susceptibility assays require isolation and passage of the virus in cell culture before testing begins. A standard inoculum of the virus is then used to infect cell cultures to which various concentrations of an antiviral agent are added. The activity of the antiviral agent against the replication of the virus is measured by one of many methods, including the inhibition of plaque formation or virus-induced cytopathic effect; a decrease in the production of viral antigens, enzyme activities, or total virus yield; a reduction in viral nucleic acid synthesis; or the inhibition of cell transformation. With each method, results are expressed as the drug concentration that causes a 50% inhibition (IC_{50}) in the growth of the virus. Phenotypic antiviral susceptibility assays have been developed and are currently in use for HSV, CMV, VZV, influenza A virus, and HIV-1. Such assays have proven to be accurate and reliable for the determination of antiviral resistance.

There are some important caveats to phenotypic antiviral susceptibility testing, however, as no standards exist, and many variables can influence the final result. Comparison of in vitro susceptibility data among different laboratories are valid only if the same assay, host cell, virus inoculum, and range of drug concentrations are used. The definition of a sensitive or resistant isolate may differ for each assay system and for different laboratories performing the same assay. Efforts to develop standardized consensus assays for HSV, CMV, and HIV-1 are currently in progress. With the exception of HSV, phenotypic antiviral susceptibility testing also can take many weeks to complete for slow-growing viruses, such as CMV, VZV, and HIV-1, and therefore may not be rapid enough to influence therapeutic decisions. The current goal for these viruses is to develop more rapid phenotypic assays. In vitro susceptibility results also may not always correlate with clinical response. HSV isolates resistant to acyclovir in vitro have responded to antiviral treatment with the drug^{36, 103} and patients with isolates that remain susceptible to acyclovir in vitro have failed therapy.³⁹ Furthermore, patients can possess a heterogeneous population of viral isolates with different in vitro susceptibilities to a given drug.⁸⁶

Genotypic Assays

Genotypic assays have been developed for the rapid detection of genetic mutations that confer viral drug resistance.^{72, 106} These assays involve using the polymerase chain reaction (PCR) for amplification of specific viral genes and direct sequencing of the amplified products to identify alterations in the viral genome known to be associated with viral resistance to a given antiviral agent. Genotypic assays have been used to screen CMV isolates for the identification of mutations in the CMV UL97 phosphotransferase gene and the UL54 DNA polymerase

gene that confer resistance to ganciclovir and/or foscarnet.^{5, 100} Also, these assays have been employed for the detection of specific mutations in the HIV-1 reverse transcriptase and protease genes following treatment of patients with antiretroviral drugs.⁵⁶ Site-directed mutagenesis can be used to produce a new recombinant virus possessing defined genetic mutations to demonstrate that the presence of a suspected drug-resistant mutation causes a change in drug susceptibility of the virus. Genotypic assays offer the distinct advantages over phenotypic assays of speed and efficiency in analyzing large numbers of viral isolates. However, these assays are cumbersome, technically demanding, and not routinely available in clinical laboratories. Also, current genotypic assays only detect known drug-resistant mutations and the results may be confounded by the presence of mutations that have no bearing on drug resistance. Phenotypic assays are still required to identify drug-resistant viruses with novel mutations for antiviral resistance.

Quantitative Monitoring of Viral Load

The development of laboratory assays to quantitate the levels of virus in infected individuals may prove to be the most valuable laboratory tool to assess disease progression, monitor the impact of antiviral therapy, and predict treatment failure and the emergence of drug-resistant viruses. Molecular methods such as quantitative competitive PCR, reverse transcriptase-PCR, nucleic acid sequence-based amplification, and branched-chain technology are now commercially available for the accurate quantitation of viral nucleic acids of HIV-1, CMV, and hepatitis B and C viruses.^{18, 85, 110} Such tests may influence the choice of initial therapy and tailor treatment regimens to reduce the risk for viral mutation and subsequent viral resistance. Sustained decreases in patient viral load during treatment suggests an appropriate antiviral effect, whereas a return of viral load to pretreatment levels is indicative of drug failure and the possible need for alternative therapy.

CONCLUSIONS

The emergence of viruses that are resistant to antiviral drugs is of growing clinical concern. Failure of patients to respond to appropriate therapy is closely related to the development of viral resistance and the transmission of drug-resistant viruses from patient to patient has been documented. Although great advances have been made in the production of new and effective antiviral agents, viruses resistant to single and multiple drugs continue to appear under the selective pressure of prolonged treatment of viral infections in the immunocompromised host. Critical to the understanding of antiviral resistance and the appropriate therapeutic management of patients is an appreciation by clinicians and laboratory personnel of the magnitude and significance of the problem and the need to identify drug-resistant viral isolates and to

define the mechanisms of antiviral resistance. This information should prove invaluable for the prudent selection and administration of antiviral agents.

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