

Antiviral Therapy

I. Introduction

The past 30 years have witnessed a revolution in the development of antiviral agents but, in general, these advances had lagged behind the development of antibacterial agents until recently. This is due to a number of challenges, which include the:

- A. Myriad number of viral agents that humans encounter.
- B. Need for knowledge of replication at the molecular level to define targets. Viruses are intracellular parasites and as such make drug targeting more difficult to avoid host toxicity.
- C. Lack of culture systems for some agents which hinders drug development (e.g., hepatitis C virus).
- D. Major approaches to drug discovery, such as high through-put screening plus 'rational' drug design, are labor intensive and expensive.
- E. Pathogenesis of certain agents makes therapy a challenge even in the face of defined targets (e.g., HIV).
- F. Clinical presentations of acute viral infections may be at peak of viral replication in vivo which makes the window for effective antiviral intervention narrow and the need to have rapid diagnostic tests available. The latter don't exist for many viral pathogens.

II. Diagnosis of Viral Infections

- A. The approach to the diagnosis of viral infections is similar to that for other infectious agents. The steps include:
 - 1. Having the proper index of clinical suspicion. The two initial questions to ask are: Is the syndrome diagnostic of a specific entity (e.g., herpes zoster or "shingles")? Is viral disease in the differential diagnosis of the presenting syndrome?
 - 2. Having the knowledge of the appropriate specimen(s) to send to secure the diagnosis. This necessitates having a proper differential diagnosis in hand and a knowledge of viral pathogenesis. The kinds of specimens to consider obtaining, depending upon the illness, are: blood, body fluids (e.g., oral or genital secretions, sputum), lesion scraping (e.g., from a vesicle), and tissue biopsies.

B. The laboratory methods to diagnose viral infections include:

1. Isolation of virus in tissue culture, animals, embryonated eggs. This approach is expensive and labor intensive and many clinical laboratories have moved away from this in favor of more rapid approaches. However, tissue culture is still utilized for many viruses, particularly herpes simplex virus which can grow in tissue culture within 24-72 hours.
2. Antigen detection in body fluids, blood, lesion scrapings, or tissue. Antigen detection is utilized routinely for analyzing skin lesion scrapings for herpes simplex virus (HSV) or varicella-zoster virus (VZV) and for the detection of respiratory viruses in pharyngeal or pulmonary specimens.
3. Nucleic acid detection in body fluids, blood or tissues. Examples of this include PCR detection of cytomegalovirus (CMV) DNA in serum, B19 parvovirus DNA in serum, herpes simplex virus DNA in cerebrospinal fluid.
4. Antibody detection. This is accomplished by detecting the presence of IgM (acute phase) antibody or a 4-fold rise in IgG titer. The latter often requires an interval of 14-21 days between acute and convalescent specimens and thus is not useful for diagnosis during the acute presentation of an illness.
5. Tissue biopsy for light microscopy supplemented by antigen and/or nucleic acid detection.
6. Electron microscopy (EM) of body fluids or tissues. This is often a “needle in the haystack” approach but can be useful when trying to determine if a viral pathogen is responsible for an elusive or new disease or syndrome. If one has an idea of which pathogen to look for, the yield of EM can be increased by applying an antibody to the specimen and immune aggregating the viral particles. This approach is commonly applied to the stool when looking for viruses associated with diarrheal outbreaks.

III. Anti-Herpessvirus Agents

The development of agents active against herpesviruses (e.g., HSV, VZV, CMV) has led the way for the antiviral field in general. Many of the principles involved in developing successful anti-herpesvirus drugs have been incorporated into other areas of antiviral drug development. Currently, there are several FDA approved antiherpesvirus compounds:

- Acyclovir
- Valacyclovir
- Famciclovir
- Ganciclovir
- Valganciclovir
- Foscarnet
- Cidofovir
- Formivirsen
- Trifluridine
- Idoxuridine

IV. Major Example: Acyclovir (ACV)

The development of acyclovir represents a watershed in the field of antiviral chemotherapy. Its characteristics are:

- a. It is an acyclic guanosine analog.
- b. It is active vs. HSV, VZV and very modestly vs. CMV.
- c. Mechanism of action. The drug is
 - Preferentially taken up by virally infected cells.
 - Monophosphorylated by virally (HSV, VZV) encoded thymidine kinases.
 - Di- and triphosphorylated (TP) by cellular kinases.
 - ACV-TP is the active moiety and acts as a competitive inhibitor of the viral DNA polymerase. Viral selectivity is also conferred by the fact that cellular DNA polymerases are much less susceptible to inhibition by ACV-TP than the viral DNA polymerases. This leads to viral DNA chain termination and the cessation of viral replication.
- d. The essentials of the pharmacology of acyclovir are as follows:
 - The drug is administered by oral, intravenous and topical routes.
 - It has an oral bioavailability 15-30%.
 - The half-life ($T_{1/2}$) 3 hrs.
 - It is primarily renally excreted.
- e. The major toxicities of acyclovir are:
 - Headache, nausea.
 - Renal dysfunction.
 - Neurologic symptoms/signs.
- f. Drug resistance
 - The development of resistance to acyclovir is mediated by mutations occurring in either the viral thymidine kinase (TK) and/or the viral DNA polymerase genes. Both TK-deficient and TK altered virus can result from mutations in the TK gene. Clinically significant infections can be caused by drug resistant HSV and VZV and are typically seen in immunocompromised hosts.

V. Anti-Respiratory Virus Agents

Progress in the treatment of respiratory viruses has been slower than that for herpesviruses, in large part due to the large number of agents which cause respiratory infections and the priority of drug discovery efforts. Within the area of anti-respiratory virus agents, progress has been greatest for anti-influenza virus drugs but treatment also exists for respiratory syncytial virus infections. The anti-respiratory virus agents that are FDA approved are:

- Amantadine

- Rimantadine
- Zanamivir
- Oseltamivir
- Ribavirin

A. Amantadine and Rimantadine

These were the first anti-respiratory virus agents developed and FDA approved. Their characteristics are:

1. They are tricyclic amines with a simple “bird cage” like structure.
2. They are active vs. influenza A viruses only at clinically achievable concentrations.
3. They share the same mechanism of action which is
 - Interference with function of the viral M2 protein. The M2 protein acts as an ion channel facilitating the hydrogen ion mediated dissociation of the matrix protein from the nucleocapsid.
4. The essentials of these drugs’ pharmacology are:
 - They are orally bioavailable.
 - Amantadine is renally excreted.
 - Rimantadine is hepatically metabolized and then renally excreted.
5. The major toxicity is
 - Neurotoxicity: amantadine > rimantadine.
6. The drugs are useful for treatment and prophylaxis of influenza A virus infections if circulating strains are susceptible.
7. Drug resistance is mediated by mutations in the M2 coding region and drug resistant virus can be transmitted person to person. Drug resistance has been increasing in circulating strains of influenza A, diminishing the utility of empirically using amantadine or rimantadine in prophylaxis or treatment.

B. Zanamivir and Oseltamivir

Recent progress in the treatment of influenza viruses is highlighted by the development of two viral neuraminidase inhibitors, zanamivir and oseltamivir. The characteristics of these drugs are:

1. They are active vs. both influenza A and B viruses.
2. Their mechanism of action is inhibition of influenza virus neuraminidase. Viral neuraminidase catalyzes cleavage of terminal sialic acid residues attached to glycoproteins and glycolipids, a process necessary for release of virus from host cell surfaces.
3. The essential pharmacology of these drugs is
 - Zanamivir. Administered by an oral inhalation device.
 - Oseltamivir. This drug is orally bioavailable and is converted from an ester prodrug to its active form. The drug is renally excreted.
4. Major toxicities
 - Exacerbation of reactive airway disease for zanamivir.
 - Nausea and vomiting for oseltamivir.
5. Indications

- Treatment of influenza A and B within 24-48 hrs of symptom onset.
 - Prophylaxis of influenza A and B virus infections.
 - N.B.: Neither drug interferes with antibody response to influenza vaccination.
6. Drug resistance
- Rarely reported prior to 2007. In 2007-08 influenza season, 8.6% of influenza A (H1N1) isolates reported to exhibit resistance to oseltamivir. This was associated with an H274Y mutation in neuraminidase and was found in persons without prior exposure to the drug.

C. Ribavirin

Ribavirin is a broadly active antiviral agent whose major clinical applications are the treatment of hepatitis C virus (in combination with interferon-alpha) and RSV. Its characteristics are:

1. It is a synthetic nucleoside analog with activity vs. a broad range of RNA and DNA viruses.
 - e.g., flavi-, paramyxo-, bunya-, arena-, retro-, herpes-, adeno-, and poxviruses
2. Its mechanism of action is complex
 - a. For influenza
 - Ribavirin-triphosphate interferes with capping and elongation of mRNA and may inhibit viral RNA polymerase.
 - b. For other agents
 - Ribavirin-MP inhibits inosine-5'-monophosphate dehydrogenase depleting intracellular nucleotide pools, particularly GTP.
3. The essential pharmacology is:
 - The drug can be administered by aerosol and oral routes.
 - It is hepatically metabolized and renally excreted.
4. Major toxicity
 - Anemia. The drug accumulates in red blood cells and shortens their life span.
5. Indications
 - Oral treatment of HCV (in combination with IFN-alpha).
 - Aerosol treatment of RSV in children.

VI. Anti-Hepatitis Agents

In recent years, substantial progress has been made in the treatment of hepatitis B virus (HBV) and hepatitis C virus (HCV) infections.

A. For hepatitis B there are four approved agents

1. Lamivudine. This is a nucleoside analog first developed for HIV. A lower dose is used for HBV (100 mg/d) than for HIV. In patients with HIV-HBV coinfection, the anti-HIV dose (300 mg/d) is used (as part of a combination regimen designed to fully suppress HIV

replication and to provide two active drugs vs. HBV – i.e., regimens designed to prevent the emergence of resistance to either HBV or HIV).

2. Adefovir dipivoxil. This is a nucleotide analog first developed for HIV but it was found to be nephrotoxic at higher doses. However, it was found to be extremely potent vs. HBV and is now approved for HBV infection at a lower (and safer) dose (10 mg/day). Adefovir is active against lamivudine-resistant strains of HBV.
3. Entecavir. This is a nucleoside analog that is active vs. HBV and has some activity vs. HIV. The usual dosage is 0.5 mg/day. In randomized trials, entecavir was found to be superior to lamivudine in treatment naïve persons. However, entecavir's activity is reduced in patients refractory to lamivudine therapy.
4. Telbivudine. This is a recently FDA approved synthetic thymidine analog with activity vs. HBV but not HIV. The usual dosage is 600 mg/day.
5. The anti-HIV drugs, emtricitabine (FTC) and tenofovir disoproxil fumarate (TDF) both have anti-HBV activity. Although they do not yet carry an FDA labeled indication for treatment of HBV, this activity must be taken into account when constructing an anti-HIV regimen in someone who is coinfecting with HBV and HIV.
6. Increasingly, HBV is being treated with a combination of two active drugs to try to prevent the emergence of resistance.

B. For hepatitis C, two drugs are used in combination: Interferon-alpha (pegylated formulation) and ribavirin.

VII. Interferons

Interferons are part of the complex cytokine repertoire and possess antiviral, immunomodulatory and antiproliferative effects.

A. Types of interferons

1. Alpha/Beta (leukocyte/fibroblast)
 - a. Coding genes located on chromosome 9
 - b. At least 24 subtypes of alpha, 1 of beta
2. Gamma
 - a. Coding gene located on chromosome 12
 - b. 1 subtype

B. The mechanisms of action of interferons are complex.

1. They do not possess inherent antiviral activity but act by inducing an antiviral state within cells. The steps involved are:
2. They first bind to specific receptors on cell surfaces.
3. Receptor associated tyrosine kinases then activated.
 - a. Tyk2 and JAK 1 for alpha and beta
 - b. JAK1 and JAK2 for gamma
4. Cytoplasmic proteins (STAT) are then phosphorylated.

- a. These move to the nucleus and bind to cis-acting elements in the promoter regions of IFN inducible genes.
 - 5. The first mechanism of viral inhibition involves the synthesis of 2'-5' oligoadenylate synthetase which is
 - a. Activated by dsRNA and then converts ATP into a series of 2'-5' oligo(A)s
 - These activate RNAase L which cleaves single stranded mRNAs
 - 6. A second mechanism involves synthesis of a dsRNA-dependent protein kinase (PKR, eIF-2 kinase)
 - a. PKR is activated by dsRNA and autophosphorylated. This in turn, phosphorylates the alpha subunit of eukaryotic initiation factor-2 which inhibits protein synthesis.
 - 7. A third mechanism is induction of a phosphodiesterase with inhibition of peptide chain elongation.
 - 8. A potential fourth mechanism is synthesis of MxA protein which can bind to cytoskeletal proteins and inhibit viral transcriptases.
 - 9. A fifth mechanism is induction of nitric oxide by gamma IFN in macrophages.
- C. The essential pharmacology of interferons are
- b. They must be given parenterally and are thus injected either intramuscularly or subcutaneously (SC).
 - c. They are inactivated in body fluids/tissues and renally excreted.
- D. Major toxicities
- d. Flu-like symptoms.
 - e. Hematologic effects, particularly leukopenia and thrombocytopenia.
 - f. Neuropsychiatric effects. Do not use interferon in persons with a history of suicidal ideations.
- E. The antiviral indications for interferon administration are
- Alpha IFN SC for HCV (in combination with ribavirin). IFN-alpha coformulated with polyethylene glycol ("pegylated" IFN) is more effective than standard IFN-alpha for treatment of HCV probably due to the more favorable pharmacokinetic profile provided by pegylation. The latter allows IFN to be dosed once weekly.
 - Intralesional for condyloma acuminata.
- F. Drug resistance can develop
- g. e.g., mutations in NS5A gene of HCV described

VIII. Passive Immunization for Viral Infections

In addition to the development of antiviral agents for specific treatment or prophylaxis of viral infections, passive administration of various immunoglobulin preparations are available for clinical use. These include:

- A. Human immune globulin

1. Prevention of hepatitis A
 2. Prophylaxis and treatment of enterovirus infections in neonates and in children with antibody deficiency
 3. Treatment of B19 parvovirus infection in immunodeficient individuals
- B. CMV immune globulin
1. Prophylaxis of CMV in solid organ transplant recipients
 2. Treatment of CMV pneumonia in combination with ganciclovir
- C. Hepatitis B immune globulin
1. Prophylaxis of hepatitis B infection
- D. Rabies immune globulin
1. Post-exposure prophylaxis for rabies (in combination with rabies vaccine)
- E. Respiratory syncytial virus immune globulin
1. Prevention of complications of RSV infection in young children
- F. Palivizumab
1. Humanized RSV monoclonal antibody
 2. Prevention of complications of RSV infection in young children
- G. Varicella-zoster immune globulin
1. Prevention of varicella infection in immunocompromised children and adults within 96 hours of exposure
- H. Vaccinia immune globulin
1. Available from CDC for complications of smallpox (vaccinia) vaccination

IX. Conclusions

The field of antiviral therapy has matured dramatically in the past 30 years with the greatest progress made for the treatment of herpesviruses, HIV, respiratory viruses and hepatitis viruses. However, preventive vaccination remains the key to the global control of viral infections.