Chapter 6

Antiviral Replication Agents

Zeinab N. Said and Kouka S. Abdelwahab

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1. Introduction

The last few decades have shown a great progress in the development of antiviral agents that were licensed for treatment of Human immunodeficiency virus (HIV), herpesviruses, hepatitis viruses and respiratory viruses. The majority of viral infections clear spontaneously and are not in need for specific medical therapy. However, antiviral chemotherapy is indicated in certain clinical situations including:

Those associated with fatal acute infections: Rabies virus, Respiratory syncytial virus, Hemorrhagic fever viruses (Lassa virus, Yellow fever, Dengue fever, Rift valley fever virus, Ebola virus) and pregnancy viral hepatitis as Hepatitis E virus (HEV) is associated with 20% fatality in pregnant females.

Human viral infections that cause persistent infections (table 1) Human viral infections associated with loss of work hours; Rhinoviruses, Influenza A virus and diarrhoea causing viruses (Calciviruses, Norwalk viruses and Astra viruses).

Family	Virus	Disease or consequence
Retroviridae	Human immunodeficiency virus (HIV)	AIDS
	Human T-cell lymphotrophic virus (HTLV)	Leukaemia
Flaviviridae	Hepatitis C virus (HCV)	Chronic hepatitis, hepatocellular carcinoma
Hepadanaviridae	Hepatitis B virus (HBV)	
Herpesviridae	Herpes simplex virus 1&2 (HSV-1) (HSV-2)	Recurrent mucocutaneous infections, encephalitis
	Varicella zoster virus (VZV)	Recurrent neurological lesions
	Cytomegalovirus (CMV)	Retinitis, pneumonia, encephalitis
	Epstein-Barr virus(EBV)	Lymphoproliferative disorders
Papovaviridae	Human Papillomavirus (HPV)	Cervical carcinoma, warts

Table 1. Common human viruses known to cause persistent infections

2. Targets for antiviral drugs

There are a number of virus-specific processes within the virus replicative cycle or inside a virus infected cell, that have proven to be attractive targets for chemotherapeutic intervention, i.e., virus adsorption and entry into the cells, reverse (RNA to DNA) transcription, viral DNA polymerization, and cellular enzymatic reactions that are associated with viral DNA and RNA synthesis and viral mRNA maturation (i.e., methylation) (De Clercq , 2001). As emphasized by Lorizate and Krausslich, (2011), viruses have to cross the host cell boundary at least twice during their replication, thus alterations of membrane lipid composition can block viral release and entry, and certain lipids act as fusion inhibitors, suggesting a potential as antiviral drugs (Lorizate and Krausslich, 2011).Most DNA viruses replicate in the nucleus and use cellular enzymes, but many DNA viruses have one or more specific viral enzymes for viral DNA replication. These enzymes are potential *targets* for effective antiviral agents. On the other hand, most RNA viruses replicate in the cytoplasm; only positive sense RNA viruses utilize the host machinery exclusively. dsRNA and negative-sense RNA viruses need to encode some, if not all virus specific enzymes for genome replication. while retroviruses have specific reverse transcriptase enzymes. Antiviral agents are effective inhibitors of these virus specific enzymes.

As viruses direct the cell machinery for effective viral replication, an effective antiviral agent must prevent completion of the viral growth cycle in the infected cell without being toxic to the surrounding normal cells (Desselberger, 1995).

The proper choice of antiviral agent relies on the selectivity index (SI) that is calculated to be the ratio of cellular toxicity to antiviral potency *in vitro* (Snoeck *et al*., 2002)

3. Definition and classification of antiviral agents

Antiviral drugs are a group of medication used for treatment of viral infections. It was formerly defined as substances other than a virus or virus containing vaccine or specific antibody which can produce either a protective or therapeutic effect to the clear detectable advantage of the virus infected host (Swallow, 1977).

Classification of antiviral agents is based on identification of a particular virus target for inhibition of a specific viral replication step.

3.1. Inhibitors of viral attachment/entry

Virus particles attach to the surface of host cells through an attachment site. Some viruses have specific attachment sites widely distributed all over the host cell membrane that recognize molecules on the surface of virus particle. This is followed by activation of an enzymatic activity that helps a change of the attachment site to allow entry of the virus into the cell. Different families of viruses have specific virus enzyme(s) whose action is to facilitate entry into the host cell. For example, the attachment site for myxoviruses is sialic

acid and the virus enzyme is neuraminidase. Sometimes two different viruses share the same attachment/entry site e.g. Coxsackie and adenovirus receptor (CAR), but the way the individual virus utilize CAR differs.

Many viruses use heparan sulphate as an attachment site (Dunn and Spear, 1989). Other cell membrane surface proteins are used as receptors by different viruses: Cluster of differentiation molecules (e.g. CD4), members of immunoglobulin (Ig) superfamily, chemokine receptors (e.g. CXCR4), glycolipids, lipoproteins, transmembrane proteins (e.g. Claudins), scavenger proteins or tumor necrosis factor (TNF) superfamily proteins. Interactions between viral surface proteins and host cell plasma membrane molecules frequently result in conformational changes that increase the efficiency of virus endocytosis/ phagocytosis and virus-mediated pathogenicity.

Antiviral drugs may act by blocking the attachment process for specific viruses. Entry of HIV involves fusion between the viral lipid envelope and host plasma membrane. Fusion inhibitors can prevent HIV direct attachment and entry: Enfuvirtide (T-20) was the first approved viral entry inhibitor (Kilby and Eron, 2003). It inhibits fusion of HIV to cell by acting as a peptidomimetic that binds to the HIV gp41 envelope protein and thus preventing its attachment to CD4+ T cells. A circulating, highly specific natural HIV-1 inhibitor, designated virus-inhibitory peptide (VIRIP) was identified by Munch et al, (2007). VIRIP blocks HIV-1 entry by interacting with the gp41 fusion peptide and it was shown that a few amino acid changes increase its antiretroviral potency by two orders of magnitude. Maraviroc (UK-427,857) is a selective CCR5 cellular receptor antagonist with potent anti-HIV-1 activity (Dorr et al, 2005, Lieberman-Blum et al, 2008). It serves to intercept viral-host protein-protein interactions mediating entry (Friedrich et al, 2011).

St Vincent et al., (2010) showed that synthetic rigid amphipathic fusion inhibitors (RAFIs) inhibit the infectivity of several otherwise unrelated enveloped viruses, including hepatitis C virus (HCV) and HSV-1 and -2 with no cytotoxic or cytostatic effects (SI > 3,000) by inhibiting the increased negative curvature required for the initial stages of fusion. On the other hand, Wolf et al., (2010) reported LJ001 as a class of broad-spectrum antivirals effective against enveloped viruses that target the viral lipid membrane and compromises its ability to mediate virus-cell fusion. LJ001 specifically intercalated into viral membranes irreversibly, inactivated virions, while leaving functionally intact envelope proteins, and inhibited viral entry at a step after virus binding but before virus-cell fusion. Also, it was recently shown that the cellular Niemann-Pick C1-like 1 (NPC1L1) cholesterol uptake receptor is an HCV entry factor amendable to therapeutic intervention. Specifically, NPC1L1 expression is necessary for HCV infection, as silencing or antibody-mediated blocking of NPC1L1 impairs cell culture-derived HCV (HCVcc) infection initiation (Sainz et al., 2012)

The second step in viral replication cycle is penetration. Enveloped viruses penetrate by fusion of the viral membrane with the cell membrane (fusion from without); however, naked viruses penetrate the cell by phagocytosis of the virion from the extracellular fluid (fusion from within). Among antiviral agents that inhibit fusion are pooled immunoglobulin, hyperimmune serum & Enfuvirtide (T-20).

3.2. Inhibitors of virus uncoating and virus genome release

In the cytoplasm cellular and virus proteases become activated by the acidic pH created inside the phagosome /endocytosome sac.Proteolytic digestion of virus capsid (naked viruses) or virus envelope (enveloped viruses) ends by complete release of virus genetic nucleoproteins.

The viral genome is released and activated by several mechanisms specific to virus families. The details are much but the end result is the start of virus replication.

Rimantadine and Amantadine specifically prevent uncoating of Influenza A (not B) virus. This is achieved by binding to virus protein M2 and blocking its action as a proton ion channel that allows acidification of the virus core needed for activation of viral RNA transcriptase. In some strains, it may inhibit virus assembly.

Amantadine is the 1-amino derivative of adamantine a complex 10- carbon compound with a cage-like structure and rimantadine is a nearly identical methyl derivative of amantadine (Hirsch et al, 1996).

Characterization of the three-dimensional structure of picornaviruses in the 1980s allowed the development of compounds targeted at the virus itself (Florea et al, 2003). Pleconaril is known to be a broad spectrum anti-picornaviral agent that binds to a hydrophobic pocket in the viral capsid inducing conformational changes, which lead to altered receptor binding and viral uncoating (Romero, 2001). Pleconaril was designed to bind the highly conserved hydrophobic binding site on VP1 protein of Picorna viruses (Hussain Basha and Prasad, 2012). Clinical studies have reported a reduction in the duration and intensity of symptoms in children and adults with enteroviral meningitis and in adults with rhinoviral respiratory tract infections treated with pleconaril. Also, pleconaril has demonstrated efficacy in the treatment of severe life-threatening enteroviral infections of the newborn and in immunosuppressed individuals. (Romero, 2001).

3.3. Inhibitors of virus replication

"All RNA viruses replicate in the cytoplasm except paramyxo viruses and retroviruses and all DNA viruses replicate in the nucleus solely except pox viruses."

RNA viruses with + ve RNA single strand genome that can act directly as virus mRNA e.g. polioviruses and hepatitis C virus use common " cellular" machines for synthesis of virus protein termed internal ribosome entry site (IRES) – mediated translation. This system of translation initiation involves entry of 40 S ribosome internally to the 5′ untranslated region (UTR) of viral RNA by a cap- independent translation using specific virus initiation factors and IRES elements required for IRES mediated translation. Because there is no RNA polymerase proofing system, several mutations occur during new viral RNA genome formation leading to quasispecies of viral RNA genome.

DNA viruses produce viral mRNA transcripts soon after the infection of a cell through host –cell enzyme, DNA dependent RNA polymerase II. DNA virus replication is semi

conservative and is very accurate since DNA polymerase checks the copied sequences (proofreading) and removes any mismatch.

3.3.1. Polymerase inhibitors

Acyclovir and other antiherpes nucleoside and nucleotide analog drugs; (Identified by the suffix –cyclovir/ -ciclovir"); Valacyclovir, Famciclovir, Penciclovir, Ganciclovir, Cidofovir (cytosine analogue). These agents interfere with virus replication and spread to new neighbouring cells by selective inhibition of an enzyme, thymidine kinase (TK) that the virus has but human cells do not, and thus interrupting the virus capability to synthesize its own DNA (table 2).CMV and EBV encode their own TK. HSV, VZV and EBV encoded TKs catalyze the phosphorylation of acyclovir to acyclovir monophosphate (ACVMP), as well as of thymidine and some other nucleoside analogue to their respective monophosphate. The markedly selective action of Acyclovir against HSV-1, HSV-2 and VZV is a consequence of several enzymatic reactions, each of which is unique for virus replication; 1) Specific activation by a virus induced TK into ACVMP, which is converted by cellular kinases to acyclovir di- and triphosphate (ACVTP), the metabolically active form of acyclovir. 2) Selective inhibition of the viral DNA polymerase by ACVTP acting as a competitor with dGTP 3) termination of viral DNA chain elongation by incorporation of ACVMP (Hirsch et al, 1996)and 4) inactivation of the viral DNA polymerase following ACVMP incorporation in the presence of dNTPs (Reardon& Spector, 1992). Also, 2-chloro-3-pyridin-3-yl-5, 6, 7, 8-tetrahydroindolizine-1-carboxamide (CMV423), showed very potent *in vitro* activity against human cytomegalovirus (HCMV). It acts on a step of the viral replicative cycle that precedes the DNA polymerase step and, most likely, coincides with the immediate early (IE) antigen synthesis (Snoeck et al, 2002).It also acts against human herpes viruses (HHV) HHV-6, and HHV-7 at low concentrations, but shows only modest activity against herpes simplex virus (HSV) HSV-1 and -2 and none against varicella-zoster virus (VZV) (Snoeck *et al*., 2002; De Bolle, 2004).

Telbivudine (LdT) is a synthetic thymidine nucleoside analogue. It is used to treat hepatitis B viral infection. It acts by blocking Viral DNA polymerase activity. Clinical trials demonstrated that telbivudine is safe and potent antiviral agent for treatment of chronic hepatitis B .It has superior efficacy compared to lamivudine(3TC) and adefovir (Lui and Chan,2008) Systematic review and meta-analysis of clinical trials showed that LdT is superior in inhibiting HBV replication and preventing drug resistance as compared to 3TC for CHB patients (Zhao et al, 2010). Adefovir(ADV) (Leung, 2005), Tenofovir and Entecavir are also nucleoside analogues with anti-HBV activity. They competitively inhibit HBV DNA polymerase ending in viral DNA chain termination after replacing viral nucleosides.

3.3.2. Nucleoside reverse transcriptase inhibitors (NRTIs)

This group includes antiviral agents that are mainly recognized for the treatment of HIV, usually in combination with other retroviral drugs (Table 3). NRTIs are the first agents that were entered into clinical trials and received approval for treatment of HIV infection (Patick & Potts, 1998).

Drug	Chemical structure	Viruses	Target
Acyclovir	Synthetic acyclic guanosine analogue [9(2hydroxyethoxymethyl)guanine] TK activated	herpes simplex virus types 1 and 2, varicella zoster virus (VZV)	viral DNA polymerase
Ganciclovir	Nucleoside analogue (acyclic analogue of guanosine) that have an extra hydroxyl methyl group on the acyclic side chain. 9-(1,3-dihydroxy- 2-propoxymethyl)guanine) virus UL97 gene-specified kinase activated (Mims et al, 2006)	Herpes viruses especially CMV	viral DNA polymerase
Cidofovir	Nucleotide analogue (S)-1-(3-hydroxy2phosphonylmethoxypropyl)cytosine) Not TK activated	CMV, HSV, Adenovirus Papillomavirus	viral DNA polymerase
Vidarbine	Nucleoside analogue (adenine) (9-β-D-ribofuranosyladenine) Not TK activated	HSV,VZV, (less effective against CMV & EBV), poxviruses, rhabdoviruses, hepadnaviruses	viral DNA polymerase
Idoxuridine	Nucleoside analogue- iodinated thymidine. Replaces thymidine in the DNA -- blocks further elongation. Virus K activated	Herpes viruses & other DNA viruses	viral DNA polymerase
Foscarnet	Non nucleoside analogue (pyrophosphate analogue); phosphonoformic acid, trisodium salt. Not TK activated	cytomegalovirus (CMV) and herpes simplex viruses types 1 and 2 (HSV-1 and HSV-2	viral DNA polymerase and HIV reverse transcriptase

Table 2. Common viral DNA polymerase inhibitors

Zidovudine

Zidovudine (azidothymidine) is a synthetic pyrimidine analogue. It is an analogue of the nucleoside thymidine in which the hydroxyl group on the ribose is replaced by an azido group (Hirsch et al, 1996). After conversion to the triphosphate by cellular enzymes, it acts as a competitive inhibitor of, and substrate for the viral reverse transcriptase. The azido group prevents the formation of phosphodiester linkages. Proviral DNA formation is blocked because AZT triphosphate is incorporated into the DNA with resulting chain termination (Mims et al, 2006, Chapter 33).

Drug	Chemical Name	Target Viruses
Zidovudine*(AZT; ZDV)	Azidothymidine	HIV
Stavudine*(d4T)	2′,3′didehydro-3′-deoxythymidine	HIV
Zalcitabine*(ddC)	2′,3′- dideoxycytidine	HIV
Lamivudine*(3TC)	dideoxy-thiacytidine analogue	HIV Hepatitis B
emtricitabine(*Emtriva)*	Deoxycytidine nucleoside analogue	HIV
Didanosine*(ddI)	2′,3′- dideoxyinosine	HIV
Abacavir (ABC)	nucleoside analog reverse transcriptase inhibitor	HIV
Tenofovir**(Tenofovir DF)	an acyclic nucleoside phosphonate (nucleotide) analog of adenosine 5'-monophosphate	HIV

* All are recognized by the "INE" suffix

** Tenofovir is a nucleoTide reverse transcriptase inhibitor

Table 3. Common Nucleoside Reverse Transcriptase Inhibitors

Lamivudine (3TC)

Lamivudine is a dideoxy-thiacytidine analogue with potent antiviral property against hepatitis B virus (Leung, 2005).Lamivudine acts as a nucleoside inhibitor of reverse transcriptase.It inhibits HBV reverse transcriptase, blocks the completion of the double stranded circular DNA before migration to the cell nucleus and prevents the infection of new hepatocytes. However, Lamivudine resistance developed after five years of monotherapy.

Tenofovir (TDF)

Tenofovir is a new nucleoside analogue with selective activity against hepatitis B virus. It was licensed in 2008 for the treatment of HBV infections in Europe and the United States (Schooley et al, 2002, Zhao et al, 2011). It is active against wild type and Lamivudine resistant HBV, both *in vitro* (Lada et al, 2004) and *in vivo* (Lacombe et al, 2005).

Systematic review and meta-analysis of clinical trials was conducted by Zhao et al (2011) to compare the efficacy of tenofovir and adefovir in the treatment of chronic hepatitis B. Meta-analysis indicated that a twelve-month TDF treatment was superior to ADV in inhibiting HBV replication in CHB patients. But there was no significant difference in the ALT normalization, HBeAg seroconversion and HBsAg loss rate.

3.3.3. Non nucleosides reverse transcriptase inhibitors (NNRTI)

Travertine, Delavirdine, Efavirenz, Nevirapine.

These drugs directly bind to different sites in the reverse transcriptase (RT) enzyme and prevent its action. They do not require phosphorylation for activation and do not compete with nucleoside triphosphates.

3.3.4. Inhibitors of RNA synthesis (RNA polymerase inhibitors)

Ribavirin: It is a synthetic purine nucleoside derivative- that resembles guanosine. Ribavirin inhibits guanosine triphosphate formation, prevents capping of viral mRNA, and blocks viral RNA-dependent RNA polymerase activity (Hirsch et al, 1996). It has got a broad spectrum antiviral activity as it inhibits replication of many DNA and RNA viruses such as HCV, Influenza A and B, parainfluenza, respiratory syncytial virus (RSV), paramyxovirus and HIV.

The combination of Interferon alpha/Ribavirin therapy was approved by the United States regulatory authorities in 1998. The clinical efficacy of this combination exceeds that of the summation of individual monotherapies (Lau et al, 2002).Four mechanisms of action of ribavirin in HCV therapy were proposed. The first line of action consists of 2 possible indirect mechanisms: (1) enhancement of host T-cell–mediated immunity against viral infection through switching the T-cell phenotype from type 2 to type 1 and (2) inhibition of the host enzyme inosine monophosphate dehydrogenase (IMPDH). The second line of action consists of 2 other hypotheses: (1) direct inhibition of HCV RNA, including NS5B-encoded RNA dependent RNA polymerase (RdRp) and (2) as an RNA mutagen that drives a rapidly mutating RNA virus over the threshold to "error catastrophe." (Lau et al, 2002).

On the other hand, specifically targeted antiviral therapy for hepatitis C (STAT-C) will probably supplement or replace present therapies. Leading compounds for STAT-C target the HCV nonstructural (NS) 5B polymerase and NS3 protease and helicase domain of the HCV NS3 protein (Belon and Frick, 2009).

3.4. Inhibitors of viral protein synthesis

All viruses use the cellular ribosomes to translate their viral mRNA. The later is translated into the structural proteins that will constitute core, envelope proteins and viral enzymes. As an example, the Enteroviruses (EV) RNA genome directs the synthesis of a single polyprotein that is autocatalytically processed into mature proteins at Gln ↓Gly cleavage sites by the 3C protease (3Cpro), which has narrow, conserved substrate specificity. These cleavages are essential for virus replication, making 3Cpro an excellent target for antivirus drug development (Costenaro et al, 2011). The crystal structure of 3Cpro from an enterovirus B, EV-93, a recently identified pathogen, alone and in complex with the anti-HRV molecules compound 1 (AG7404) and rupintrivir (AG7088) was determined by Costenaro et al (2011). They found that the EV-93 3Cpro adopts a chymotrypsin-like fold with a canonically configured oxyanion hole and a substrate binding pocket similar to that of rhino-, coxsackie- and poliovirus 3C proteases (Costenaro et al, 2011). Collectively, neuraminidase enzyme regulates the synthesis of viral and cell membrane glycoprotein during Influenza virus A and B replication, which characterizes the enzyme as a target of viral protein modification inhibitors (neuraminidase inhibitors).

Other examples of viral proteins synthesis inhibitors are Fomivirsen and Interferon. Fomivirsen is an oligonucleotide that binds to CMV mRNA and blocks its replication and thus inhibits the synthesis of proteins that are essential for production of infectious CMV. It

is a potent and selective antiviral agent for cytomegalovirus retinitis (Geary et al, 2002). Interferons are a group of virus induced proteins that interrupts new viral protein formation by several mechanisms .They possess direct complex intracellular antiviral, antiproliferative, and immunomodulating activities (Lau et al, 2002). IFN-α and -β have got antiviral activity whereas IFN-γ is predominantly immunomodulatory. rIFN-α and rIFN-β are approved for treatment of HCV, HBV, HPV and HHV-8 (Kaposi sarcoma) infections.

Gene targeting studies have distinguished four main effector pathways of the IFN-mediated antiviral response: the Mx GTPase pathway, the 2', 5'-oligoadenylate-synthetase-directed ribonuclease L pathway, the protein kinase R pathway and the ISG15 ubiquitin-like pathway. These effectors pathways individually block viral transcription, degrade viral RNA, inhibit translation and modify protein function to control all steps of viral replication (Sadler &Williams, 2008).

The aim of HCV/ HBV treatment is to develop a sustained decline of viral load by inhibition of viral replication, allowing CTL-derived cytokines to reduce the number of hepatocytes supporting viral replication by direct killing and also improvement of liver histopathology by the decline of HBV/HCV infected hepatocytes This will decrease fibrosis and hepatocytes regeneration with subsequent reduction of liver cirrhosis and thus prevention of progression to hepatocellular carcinoma.

As suggested by Sainz et al, (2012), optimal HCV therapy will probably require a combination of antiviral targeting multiple aspects of the viral lifecycle. Recently, high rate of sustained virologic response was achieved when two direct-acting antiviral agents (NS5A replication complex inhibitor daclatasvir and the NS3 protease inhibitor asunaprevir) were combined with peginterferon alfa-2a and ribavirin for treatment of HCV chronic hepatitis patients (Lok et al, 2012).

Several drugs are recommended for treatment of patients with chronic hepatitis B (CHB).These anti HBV drugs are used to compose combinational therapy with the addition of interferon sometimes to delay drug resistance. These drugs can be divided into two main groups based on their mechanism of action, namely immunomodulatory drugs like alpha interferons and antiviral drugs including lamivudine(LAM), telbivudine(LdT), entecavir(ETV), adefovir(ADV), and tenofovir(TDF) (Zhao et al, 2011).Interferon and Lamivudine have been the only approved agents for a while. The approval of Adefovir in 2002, Pegylated Interferons and Entecavir in 2005 opens up more choices and chances (Leung, 2005).

3.4.1. Protease inhibitors (PIs): (inhibit the post-translational events)

Various drugs recognized by "NAVIR" suffix are known to have the same mode of action; *Amprenavir, Saquinavir, Darunavir, Atazanavir, Ritonavir, Tipranavir Indinavir, Nelfinavir.*

This group of drugs acts by preventing the activity of cellular/viral proteases enzymes. Proteases are valid targets for antiviral agents as they are essential for the production of

mature infectious virus particles. Molecular studies have indicated that viral proteases play a critical role in the life cycle of many viruses by affecting the cleavage of high-molecular-weight viral polyprotein precursors to yield functional products or by catalyzing the processing of the structural proteins necessary for assembly and morphogenesis of virus particles (Patick & Potts, 1998).

Several studies elaborated the value of protease inhibitors for the treatment of a lot of RNA and DNA viruses; HIV, HCV, Picorna viruses, RSV, Herpes viruses, Rota virus & severe acute respiratory syndrome virus (SARS). HIV protease inhibitors have emerged as potent antiretroviral chemotherapeutic agents that, in combination with RTIs, have resulted in prolonged suppression of viral replication (Patick & Potts, 1998). Also, in HCV treatment, direct acting antivirals (DAA), in clinical development include NS3-4A protease inhibitors (two of which, telaprevir and boceprevir, have recently been approved for treatment of HCV genotype 1 infection in combination with pegylated interferon-α and ribavirin (Pawlotsky, 2012). Replication of picornaviruses and coronaviruses requires 3Cpro (3C protease) and 3CLpro (3C-like protease) respectively, which are structurally analogous (Ramajayam et al, 2011). A group of common inhibitors against 3C (pro) and 3CL (pro) were found recently (Wang and Liang, 2010).

3.4.2. Integrase inhibitor: Raltegravir

Integrase is an essential HIV-1-specific enzyme that is an active target for antiretroviral drug development. The drug specifically inhibits strand transfer, one of the three steps of HIV integration into the host DNA (Katlama and Murphy, 2009) and thus prevents human immunodeficiency virus from multiplying in the host.

Inhibition of HIV replication initially targeted viral enzymes, which are exclusively expressed by the virus and not present in the human cell (Sierra-Aragón and Walter, 2012).

Table 4 illustrates Common FDA approved antiviral agents for the treatment of HIV infection (www.fda.gov/.../hivandaidsactivities/ucm118915.ht.)

Analogue to HCV therapy, combination therapy for treatment of HIV reduces HIV replication with subsequent drop in viral load. Two NRTIs in combination with the NNRTI or PI drugs have had a dramatic effect on progression to AIDS and led to the term Highly Active Anti-Retroviral Therapy (HAART) (Mims et al, 2006- chapter 21). The combination includes:

Nucleoside/ Nucleotides Reverse Transcriptase Inhibitors (NRTIs) such as Tenofovir and Abacavir

Non-Nucleoside/ Nucleotides Reverse Transcriptase Inhibitors (NNRTIs) such as Efavirenz and Nevirapine

Integrase Inhibitors such as Raltegravir

Protease Inhibitors (PIs) such as Darunavir and Atazanavir

Fusion and Entry Inhibitors such as Enfuvirtide and Maraviroc.

Name	Mechanism of action
Lamivudine,zidovudine,emtricitabine, abacavir, stavudine,didanosine, zalcitabine	Nucleoside Reverse Transcriptase Inhibitors (NRTIs)
tenofovir disoproxil fumarate	Nucleotide reverse transcriptase inhibitor (NtRTI)
Rilpivirine,delavirdine,etravirine,efavirenz, nevirapine,nevirapine	Nonnucleoside Reverse Transcriptase Inhibitors (NNRTIs)
Amprenavir, tipranavir, lopinavir (combined with ritonavir at a 4/1 ratio),indinavir,ritonavir,darunavir, nelfinavir, atazanavir	Protease Inhibitors (PIs)
enfuvirtide, T-20	Fusion Inhibitors
maraviroc	Entry Inhibitors - CCR5 co-receptor antagonist
raltegravir	integrase inhibitors

(www.fda.gov/.../hivandaidsactivities/ucm118915.ht.)

Table 4. Common FDA approved antiviral agents for the treatment of HIV infection

3.5. Inhibitors of viral exit (release)

Viral release is done by single burst releasing millions of new viral particles from infected cells or by slow process of budding through the plasma membrane allowing the infected cell to survive for several days while supporting viral replication and release. In general, lytic viruses (e.g: polio) are released by lysis and death of the cell. Others (e.g. influenza, HIV, and measles) escape by budding from the cell surface.

3.5.1. Neuraminidase inhibitors

Oseltamivir and Zanamivir are antivirals used to treat and prevent influenza (Jefferson et al, 2012). They Inhibit neuraminidases produced by influenza A and B (enzyme which cleaves the interaction between sialic acid cell surface receptors and viral proteins and surface proteins of infected cells and thus allow for release of virions) Therefore, these drugs interfere with the release of influenza virus from infected host cells.

Table 5 sums up antivirals that are available commercially, used by physicians and approved by international and national regulatory authorities.

Virus	Anti-Viral Drugs
Human immunodeficiency virus	22 approved agents
Hepatitis C virus	pegIFN, Ribavirin
Hepatitis B virus	Interferon-alpha (pegylated), Lamivudine, adefovir
Herpesviruses	Acyclovir,famciclovir,valacyclovir,ganciclovir, cidofovir, formivirsen,valganciclovir
Influenza	Amantadine, rimantadine,zanamivir, oseltamivir
Respiratory syncytial virus	Ribavirin, Palivizumab
Picornaviruses	pleconaril
Papillomaviruses	IFN(intra-lesional), ?cidofovir, Fluorouracil
Rhinoviruses	Tremacamra (rsICAM-1)

Table 5. Master Anti-Viral Drugs

4. Antiviral from plants

Plants and plants extracts have been used chiefly as traditional medicine for centuries even before the active principles in the plant products could be elucidated through the improvements in science and technology. The World Health Organisation (WHO) has estimated that perhaps 80% of the world's population rely on traditional medicine for the treatment of infectious diseases (Abonyi et al, 2009).

Several naturally occurring dietary flavonoids including quercetin, hesperetin, and catechin were previously studied *in vitro* in cell culture monolayers using viral plaque reduction technique and proved to be effective on the infectivity and replication of HSV-1, poliovirus type 1, parainfluenza virus type 3 (Pf-3), and respiratory syncytial virus (RSV). Quercetin caused a concentration-dependent reduction in the infectivity of each virus. Hesperetin had no effect on infectivity but it reduced intracellular replication of each of the viruses. Catechin inhibited the infectivity but not the replication of RSV and HSV-1 and had negligible effects on the other viruses (Kaul et al, 1985).

We previously had the opportunity to evaluate extracts of five different herbal plants for hepatitis A (HAV) antiviral activities by plaque reduction assay. These plants were anise, chamomile, liquorice, nigella and thyme. A fast growing HAV-10 reported to be cytopathic

for *Vero* cell culture was used where the plant extracts were screened for anti-infective, protection and antireplicative activities. The rates of the anti-infection studies were arranged in a decreasing order as follows: anise> liquorice > chamomile > thyme>. The rates of the protective antiviral activities were found to be as follows: chamomile >liquorice> thyme > anise. The rate of the anti-replication effect was ordered in the following decreasing order: thyme > liquorice > chamomile > nigella. Thus anise extract was devoid of any anti-replication activity, whereas nigella extract was devoid of any protective activity against HAV infection i*n vitro* (Omran et al, 2001)

Also, another Egyptian study evaluated the antiviral activities of the essential oils of the fresh leaves of 3 *Melaleuca* species; *M. ericifolia*, *M. leucadendron*, and *M. armillaris* against *Herpes simplex virus* type 1 (HSV-1) in African green monkey kidney cells (Vero) by a plaque reduction assay. It was found that the volatile oil of *M. armillaris* was more effective as a virucidal (up to 99%) than that of *M. leucadendron* (92%) and *M. ericifolia* (91.5%) (Farag et al, 2004).

Amylose extracted from Grateloupia filicina have antivirus activity in the stage of HSV-2 binding, adsorption and ingression with Vero cell (Zhu et al, 2006).

Also, it was shown by Verma et al, (2009) that Picroliv or Kutkin of Picrorhiza kurroa which constitute an important component of many Indian herbal preparations has anti-viral and immune-stimulant activities.

Screening of the antiviral activity of oil extract of Balanites aegyptiaca (Balantiaceae) fruits against Herpes simplex virus type -1 in African green monkey kidney cells (Vero) by a plaque reduction assay, illustrated that the oil had virucidal activity (58.3%) against Herpes simplex virus type 1 at concentration 50μg/ml compared with acyclovir (60%) at the same concentration (Al Ashaal et al, 2010).

The antiviral activity of Balanites aegyptiaca herb was also reported against HIV/AIDS (Chaudhry and Khoo, 2004).

We also have the experience of testing extracts from Egyptian medical plants for their potential antiviral activity at different stages of viral replication. The anti-influenza activity of hydro-alcoholic extracts from; Cleome droserifolia, Justicia ghiesbreghtiana, and Thunbergia grandiflora against a fast growing Influenza "A" reference strain (H3N2) in replicating Madin–Darby Canine kidney (MDCK) was performed. Amantadine was used as reference anti-influenza virus drug. Three antiviral assays were used; Anti-infectivity, protective and anti-replication mechanisms, reduction in the number of plaques formed by the virus in MDCK cells treated with maximum non toxic dose (MNTD) of each plant extract was analyzed. Justicia extract showed the highest significant inhibition of influenza virus infectivity .On the other hand, when MDCK cells were pretreated with cleome extract for 48 hours before infection with influenza virus, it showed the highest significant anti-influenza virus activity. Whereas, when cleome extract was added to MDCK infected with influenza virus after 60 min of infection, it induced significant inhibition in influenza virus type A replication in a dose dependent relation. Thunbergia extract had the least antiviral

activity. Phytochemical screening tests showed that all of the studied extracts contain tannins but flavonoids are present in the Cleome and Justicia extracts only. Two major compounds of cleome extract; Isorhamnetin-3-0-B glucopyranosyl-7-0 -L-rhamnopyranoside and quercetin -3-0-B – glucopyranosly-7-0-L rhamnopyranoside were studied for their antiviral activity by the three different assays. The results proved that isorhamnetin produced inhibition of influenza virus infectivity stronger than that produced by the total cleome extract (Elkosy et al, 2005).

Antiviral activity of dandelion extracts against influenza viruses was reported by Wen et al, (2011). Mechanisms of reduction of viral growth in MDCK or A549 cells by dandelion involve inhibition of virus replication. Dandelion extracts inhibited infections in Madin-Darby canine kidney (MDCK) cells or Human lung adenocarcinoma cell line (A549) of PR8 or WSN viruses, as well as inhibited polymerase activity and reduced virus nucleoprotein (NP) RNA level. The plant extract did not exhibit any apparent negative effects on cell viability, metabolism or proliferation at the effective dose (Wen et al, 2011).

In general, the medicinal plants are potential antiviral agents that are locally available, relatively cheap, can be tested for safety and non toxicity and culturally acceptable to the community.

5. Conclusions

Potent antiviral agents are on the increase leading to improved patient management. Viruses replicate inside live nucleated cells in different steps using cellular machinery, but sometimes virus specific enzymes are used. This allows for the selection of virus specific molecules as targets of antivirals. An effective antiviral therapy depends greatly on its ability to block viral entry or viral exit from infected cell or inhibiting active viral replication steps. Proper understanding of these steps at molecular level and the use of advanced *in silico* design for development of antivirals that specifically react with viral target molecule may provide new insight for their potential activity and prophylaxis against viral infections.

Author details

Zeinab N. Said and Kouka S. Abdelwahab
Faculty of Medicine (for Girls)-Al-Azhar University, Cairo, Egypt

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