Antiviral drugs.

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Antiviral drugs

E.H.H. Wiltink and R. Janknegt

Introduction

Viruses have too simple a structure to multiply themselves. For multiplication, a virus invades a cell, using the biochemical mechanisms of this cell to make new viral proteins and genetic material. So, virus and host cell are intimately connected and an effective antiviral drug must be able to distinguish the virus from the host cell. In the last twenty years there has been a growing understanding of viral multiplication, which has allowed us to develop new drugs in the battle against viral infections.

The development and marketing of aciclovir in 1981, was a break-through in antiviral therapy. Aciclovir is effective in a number of viral herpes diseases with only limited adverse effects. Mortality and morbidity of herpes virus infections have decreased dramatically.

Together with the increasing use of immunosuppressive drugs, bacterial and viral infections have become a more pronounced problem and require the development of new drugs. This proces was accelerated by the human immuno-deficiency virus (HIV) outbreak, which required anti-HIV drugs and antiviral drugs for viral infections secondary to AIDS. At this moment enormous amounts of money and time are spent in the research for new antiviral drugs.

The aim of this review is to look at the available antiviral drugs, their use and disadvantages and to discuss the therapy of common viral diseases and the possibilities for the near future.

Modes of infection

In developing new antiviral drugs, it is important to understand the modes of viral infection. One can determine a number of steps when a virus is infecting a cell. Intervening in one of these steps gives the opportunity to control a viral infection. The following steps can be distinguished (Fig. 1): the virus binds to the surface membrane of the cell (1: attachment), enters the cell (2: penetration) and sheds its protein coat (3: uncoating). The released genetic material (DNA or RNA) activates the cellular biochemical machinery, after which viral multiplication in the nucleus or cytoplasm can occur (4: multiplication). When the new viral genome formation is finished and viral proteins are formed (5: protein synthesis), the genetic material is coated by these proteins (6: assembly). The virus is released and is able to infect other cells (7: release).

The viral genetic material may be a single-strand or double-strand molecule of either DNA or RNA (DNA or RNA virus), which only carries a few genes, coding for enzymes, regulatory proteins, neccesary to interfere with the host cell in order to multiply itself and its structural proteins. In the case of a DNA virus the first step in multiplication is transcription to mRNA. In the RNA virus there are two possibilities: RNA directly behaves like mRNA or after transcription by viral transcriptase to mRNA.

Viral infections can express themselves in several ways. In a so-called 'lytic' infection the host cell bursts when releasing the newly formed particles and dies. Lytic infections are characterized by a rapid spread throughout the population (*e.g.* common cold and polio). In a 'persistent' or 'chronic' infection the virus is released gradually. The host cell is not always killed and is able to reproduce itself whereby the viral genetic material is also multiplied. Examples of this kind of infection are hepatitis B viruses, human immuno-deficiency virus and human leukaemia viruses. This type of infection can proceed for many years without causing symptoms. The third kind of infection is the 'latent' infection. The virus is not reproducing itself, but the genetic material is integrated in the cellular genome or in episomes. During cell division the genes of the virus are reproduced together with the genes of the cell and transmitted to the daughter cells. A latent virus is characterized by periods of active viral replication. Many of the herpes viruses occur as latent infections: herpes simplex type 1 and 2,

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Abstract

There are only a limited number of effective, non-toxic antiviral drugs for clinical use, whereas there is a great need for such drugs. Especially for the treatment of patients infected with the human immuno-deficiency virus (HIV) anti-HIV drugs are required. At the same time viral infections secondary to AIDS cannot yet be treated effectively. An increasing problem is the development of virus strains resistant to the available drugs. At this moment a great effort is made in the research for new antiviral drugs. In this article the available antiviral drugs are reviewed. Their antiviral properties, mechanism of action, clinical use, pharmacokinetic properties and side-effects are discussed. Some attention is paid to the future directions in the search for new anti-HIV drugs.

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varicella zoster, Epstein-Barr and cytomegalovirus [1].

Modes of treating infections

Besides the problem of distinguishing virus from host cell, the fact that symptoms of the infection only appear after some time, after replication and tissue damage have been going on for a while, provides a second problem. A first contamination frequently occurs without serious symptoms or even unrecognized, whereafter the virus can be latent for many years before expressing itself. Moreover, virus identification is difficult and time-consuming, where quick therapeutic intervention is needed to avoid spread of the virus. The possibilities for therapeutic interventions are summarized in Table 1.

Figure 1
Viral infection at host–cell level

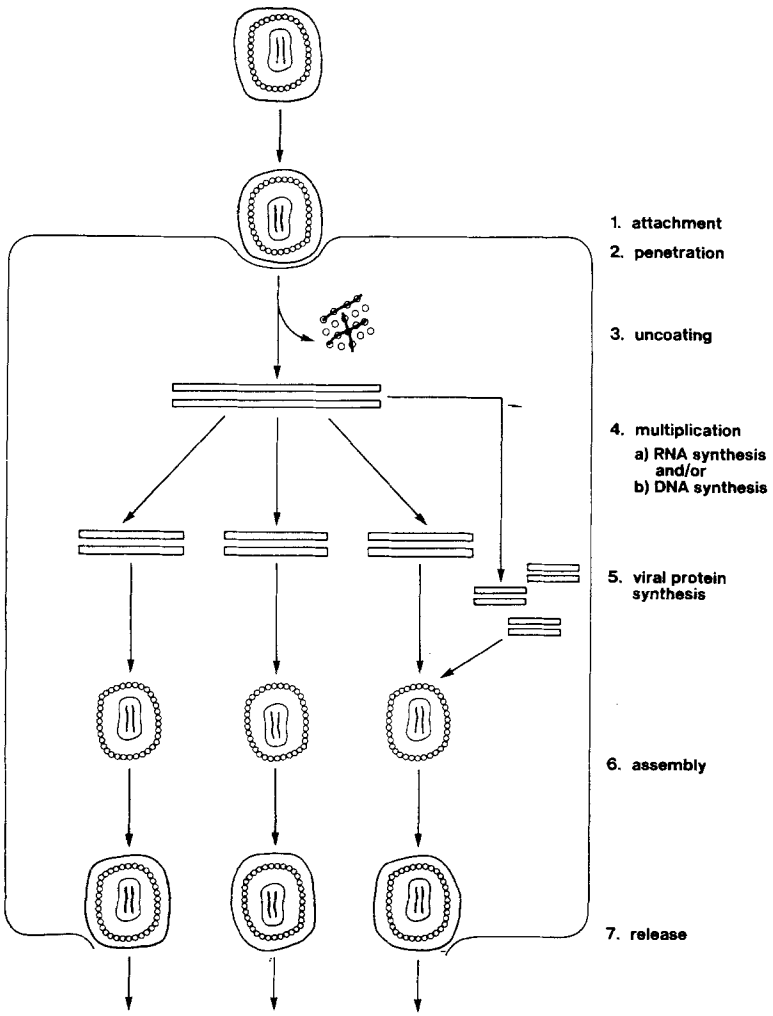


Table 1
Mechanism of action of antiviral drugs

1 attachment
vaccination, immunoglobulins, inosiplex (interferons)
2 penetration
(amantadine, rimantadine, tromantadine)
3 uncoating
(amantadine, rimantadine, tromantadine)
4 replication
– RNA synthesis
(amantadine, rimantadine, tromantadine)
foscarnet, ribavirin
– DNA synthesis
idoxuridine, trifluridine, aciclovir, ganciclovir, foscarnet
zidovudine
5 viral protein synthesis
(interferons)
6 assembly
metasizon (interferons)
7 release
(interferons)

Attachment

The best method of dealing with viral infections is to avoid them. This can be done by means of stimulating the immunological system to produce antibodies against the virus. Vaccination offers such an opportunity, using passive or active immunization. As a rule, vaccination is used prophylactically and in some cases therapeutically (rabies virus). A number of vaccines are included in the Dutch vaccination programme, such as mumps, measles, rubella and poliomyelitis, while others are used in patients at risk: influenza or hepatitis B. Such vaccines stimulate the production of antibodies against these viruses. This leads to protection ranging from a couple of years to the whole life-time.

Immuno-globulins (antibodies) are proteins, used for passive immunization. These antibodies are specific immuno-globulins with a high concentration of antibodies against one or more exactly described antigens. They can either be used therapeutically or prophylactically. Examples are cytomegalovirus-immuno-globulin, hepatitis B-immuno-globulins and tetanus-immuno-globulin. So formed (vaccines) or administered (immuno-globulins) antibodies bind to the virus particles and prevent the virus from invading a cell. Strictly spoken these vaccines and immuno-globulins cannot be considered as real drugs, but they are useful in certain groups of people at risk.

Only one drug with immuno-modulating properties is in use: inosiplex (inosine pranobex). Interferons have at least partly immuno-regulating functions, but they are not strictly antiviral agents.

Penetration

Penetration into the host cell is the next step in the development of viral infections. It is possible but not actually proven that amantadine, rimantadine and tromantadine interfere with penetration.

Uncoating

Amantadine, rimantadine and tromantadine inhibit the uncoating of the viral genome. Perhaps they interfere with the transcription to mRNA.

Replication

Most of the antiviral drugs interfere with the synthesis of the genetic material of the new virus. There is a difference between inhibiting RNA synthesis (ribavirin, maybe amantadine)

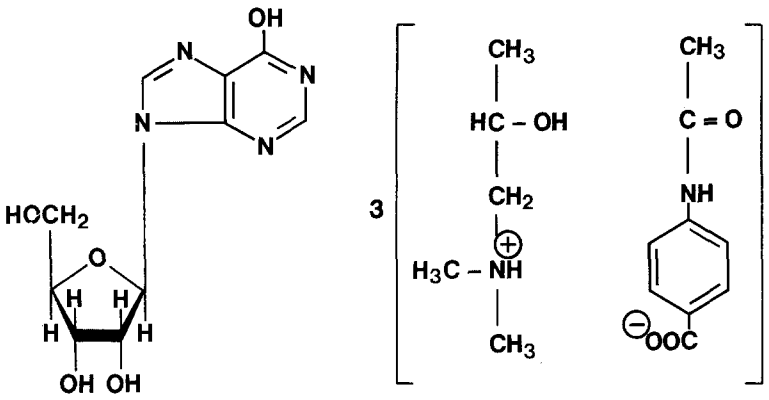


Figure 2
Structure of inosiplex

and DNA synthesis (idoxuridine, trifluridine, aciclovir and ganciclovir).

Protein synthesis

Interferons could play a role in the protein synthesis of the new virus. On the one hand they break down replicating virus, on the other hand they activate an enzyme capable of interrupting the protein synthesis [2].

Assembly

Metasizon is able to interfere with the assembly of the virus, but it is also involved in inhibiting RNA synthesis.

Antiviral drugs

Inosiplex

Inosiplex is the *p*-acetamidobenzoic salt of *N,N*-dimethylamino-2-propanol and inosine in a 3:1 molar ratio (Fig. 2). It has a weak antiviral and anti-tumour activity. An immuno-modulating effect probably plays a role, but inosiplex also increases cell ribosomal RNA and protein synthesis, while simultaneously inhibiting the use of cell ribosomal RNA for viral replication.

Inosiplex is claimed to be effective in several viral infections, such as mucocutaneous herpes simplex, sub-acute sclerosing panencephalitis, influenza, herpes zoster and type B hepatitis, but various reports are conflicting [2 3].

Very little has been published on the pharmacology and pharmacokinetics of inosiplex. The usual daily oral dosage is 25 to 100 mg/kg or 3 to 6 g divided into 4 to 6 doses. The elimination half-life is 50 min. Uric acid is the major excretion product in the urine [2 3]. This leads to transient increases of uric acid concentrations in serum and urine, so, one should be cautious in patients with renal failure, gout or hyperuricaemia.

In the treatment of first-attack genital herpes, as well as in the treatment of frequently recurring genital herpes aciclovir is more efficacious than inosiplex [4].

There is limited experience with this drug in patients with AIDS [5]. It is suggested that combined therapy with inosiplex and zidovudine makes it possible to lower the dose of zidovudine and to prolong the dose interval. Moreover, inosiplex enhances the immunological response of the host [6]. Recently, it was described that treatment with inosiplex could delay progression to AIDS in a 24-week period in patients with HIV infection, but without manifest AIDS. However, the duration of this beneficial effect, the optimal dose, and the mode of action of inosiplex has to be clarified [7]. It is too early to establish the therapeutical value of inosiplex in viral infections.

Inosiplex has been well-tolerated without serious reported side-effects.

Amantadine, rimantadine and tromantadine

The mechanism of activity of amantadine, rimantadine and tromantadine (Fig. 3) is not yet elucidated. It is possible that they prevent the penetration of the virus into the cell, but it is more likely that they interact with the uncoating of the virus. Amantadine and rimantadine are only active against influenza A and minimally active against influenza C and B. *In vitro* all subtypes of influenza A virus are sensitive to amantadine and rimantadine at levels of 0.2-0.4 mg/l [8 9].

Recently, it was published that rimantadine was ineffective in protecting members of the same household from influenza A infection. Moreover, a major problem consists in the rapid selection and apparent transmission of drug-resistant influenza A viruses [10].

The recommended dose is 200 mg per day (less in the elderly or children under 10 years old) once a day or in 2 divided doses. Amantadine is well-absorbed after oral administration, reaches peak levels 2-4 h after a 200 mg dose and has a serum half-life of 14 h. It is excreted by the kidney (95% of a dose unchanged). In renal failure the dose should be reduced according to creatinine clearance. Central nervous system side-effects of amantadine occur in 11% of patients using 200 mg per day.

Low-dose (100 mg a day) amantadine for 8 days has been reported to be effective in both prevention and therapy of influenza illness [11].

Rimantadine is well-absorbed and peak levels occur 3-8 h after a 200 mg oral dose. The serum half-life is about 30 h and the drug is extensively metabolized. 80% Of the administered dose is excreted in the urine as hydroxylated metabolites [8 9].

The clinical use of amantadine and rimantadine is limited to the prophylaxis (with or without concomitant vaccination) and therapy of influenza A in certain high-risk groups of patients including adults and children with chronic cardiovascular or pulmonary disease or patients with immunological disorders. People who have extensive contact with high-risk patients also have to be considered for receiving the drug. Amantadine is prophylactically effective in 70-90% of experimentally induced and naturally occurring in-

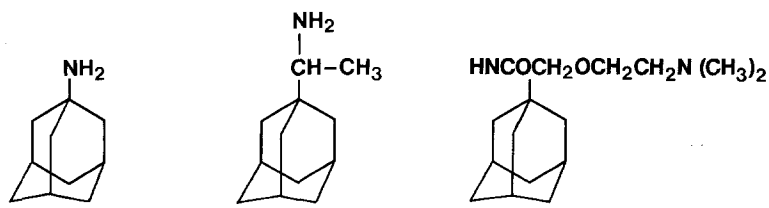


Figure 3
Structures of amantadine (left), rimantadine (middle) and tromantadine (right)

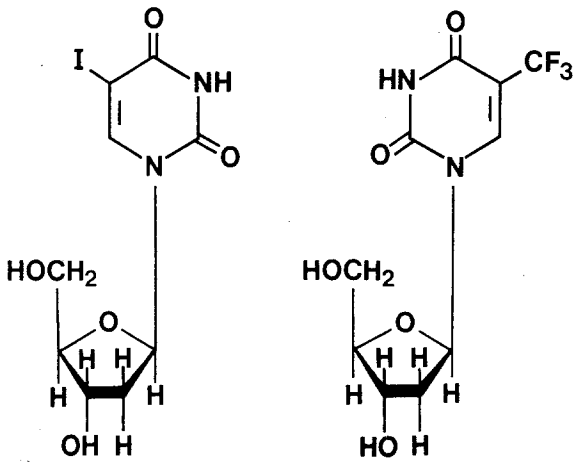


Figure 4
Structures of idoxuridine (left) and trifluridine (right)

fluenza A. Rimantadine is used extensively in the USSR. It has greater activity and fewer side-effects than amantadine. It has been submitted to the Food and Drug Administration. Both drugs also have been demonstrated to be active in the therapy of influenza A virus [8].

Tromantadine acts similarly to amantadine. It is only used locally in skin and eye infections caused by herpes simplex virus. Tromantadine is available as a 1% ointment. Contact dermatitis frequently occurs [12].

Idoxuridine and trifluridine

Idoxuridine and trifluridine are both halogenated thymidine analogues (Fig. 4). They are incorporated instead of thymidine during the building of viral DNA. They can only be used topically, because they are too toxic for oral or parenteral use. Trifluridine is used in ocular infections caused by herpes simplex virus. It seems to be more effective than idoxuridine. Idoxuridine is available for use in eye and skin infections caused by herpes simplex, vaccinia and varicella zoster virus. Its use, dissolved in dimethylsulfoxide, in skin infections is controversial [2]. Aciclovir has become drug of choice for these infections.

Vidarabine

The first antiviral agent available for parenteral use was vidarabine. Vidarabine is a purine nucleoside (Fig. 5). Intracellularly vidarabine and its main metabolite arabinoside hypoxanthine are phosphorylated to their corresponding monophosphate, diphosphate and triphosphate, which competitively and selectively inhibit virus-controlled DNA polymerase [13]. Vidarabine is active against pox virus and rhabdovirus but most of all, herpes viruses: herpes simplex 1 and 2, varicella zoster, cytomegalovirus and vaccinia. It can reduce herpes simplex virus plaque formation for 90% at 10 mg/l [13].

Vidarabine given intravenously is quickly deaminated to its principal metabolite arabinoside hypoxanthine, which appears promptly in the plasma with a peak of about 3 mg/l at the end of an infusion of 10 mg/kg over a 12-h period. The serum half-life of arabinoside hypoxanthine is about 5 h. Most of the drug is excreted in the urine as arabinoside hypoxanthine, which accounted for 40 to 50% of the dose; vidarabine appears in the urine unchanged for 2% of the dose [14]. Arabinoside hypoxanthine is much less active than vidarabine itself, but synergism occurs between arabinoside hypoxanthine and vidarabine [9].

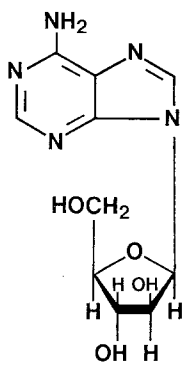


Figure 5
Structure of vidarabine

Vidarabine is mainly used as an eye ointment in the treatment of herpes simplex keratoconjunctivitis. For systemic use, it has been almost totally replaced by aciclovir, which is less toxic and more effective. Vidarabine has the disadvantage of poor solubility in water, so large amounts of fluids are needed for administration. The better soluble vidarabine monophosphate ester is far less effective.

The most important adverse reactions involve the gastro-intestinal tract (10-15%). These adverse effects seldom require termination of treatment. When the dosage exceeds 15 $mg \cdot kg^{-1} \cdot d^{-1}$ central nervous system adverse effects and bone-marrow depression have been observed.

Vidarabine may be important when drug resistance should occur in the herpes viruses to aciclovir or the commonly used antiviral agents. The suggested dose is 15 mg/kg bodyweight a day for 10 days [2 9 13].

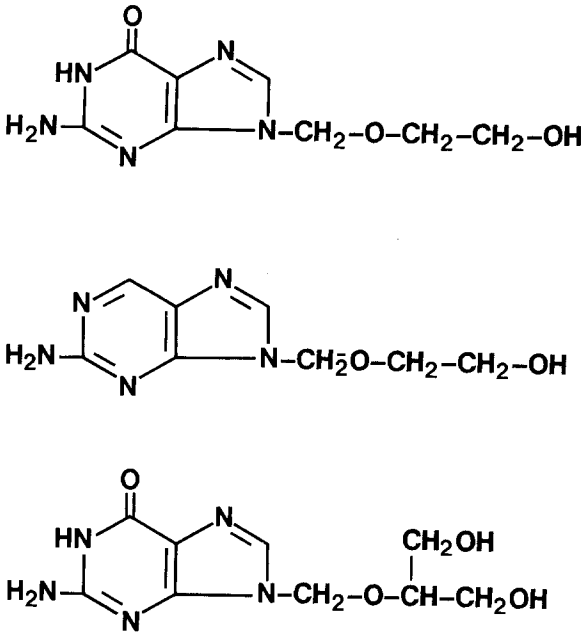
Aciclovir and desciclovir

Since its introduction in 1981, aciclovir has been one of the mainstays in the treatment of herpes simplex virus (HSV) and varicella zoster virus infections, because it is both an effective and a safe drug. Aciclovir is an acyclic analogue of guanosine (Fig. 6). To exert its antiviral activity it is necessary that aciclovir is phosphorylated to aciclovir triphosphate (Fig. 7). In the host cell aciclovir is converted by a virus-specified thymidine kinase to aciclovir monophosphate. This first step in the metabolism of aciclovir explains directly its limitations in therapeutic use. Only herpes simplex virus and varicella zoster virus are able to induce thymidine kinase. The clinical experiences with the treatment of the other important herpes viruses, Epstein-Barr virus and cytomegalovirus, are disappointing. There are no data about the activity of aciclovir against the newly discovered human herpes virus-6 (HHV-6).

Aciclovir monophosphate is phosphorylated to aciclovir diphosphate and triphosphate by cellular enzymes. Aciclovir triphosphate inhibits the viral DNA polymerase and/or is a chain-terminator of viral DNA. As phosphorylation starts by means of a virus-specific thymidine kinase, aciclovir is a safe drug: in the non-infected cell there will be little phosphorylation and aciclovir will hardly be converted to the active form. This means that aciclovir has a high therapeutic index and few side-effects.

Unfortunately, aciclovir is only active against replicating viruses. It does not eliminate latent herpes viruses [2 15]. The *in vitro* median ID50 (50% inhibitory dose, concentration required to inhibit viral replication by 50%) for herpes simplex virus 1 is 0.1 mg/l (range 0.02-41.5), for herpes simplex virus 2 0.4 mg/l (0.13-83) and for varicella zoster virus 2.6 mg/l, as measured by the

Figure 6
Structures of aciclovir (top), desciclovir (middle) and ganciclovir (bottom)

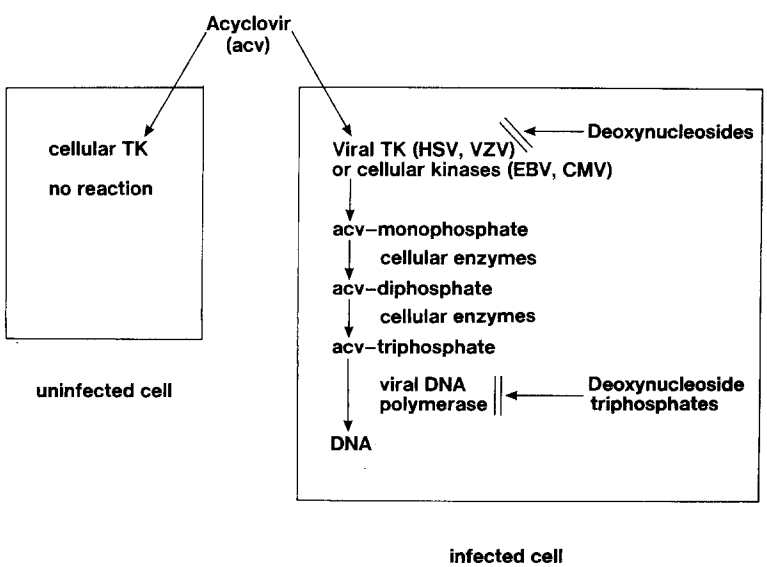


plaque reduction assay. The *in vitro* sensitivity of human cytomegalovirus varied from 18.2 to more than 200 mg/l (median 63.1 mg/l) and the *in vitro* sensitivity of Epstein-Barr virus is about 1.5 mg/l [15].

An increasing problem in antiviral therapy is the development of aciclovir-resistant strains of varicella zoster and herpes simplex in patients with AIDS. Erlich *et al.* describe 12 AIDS patients with ulcerative mucocutaneous laesions who failed to heal with high-dose aciclovir therapy. Aciclovir-resistant HSV-2 was recovered [16]. Jacobsen *et al.* report about four patients with HIV infection where varicella zoster, resistant to aciclovir *in vitro*, was isolated from skin laesions [17].

The bioavailability of oral aciclovir is only about 15-20%. The volume of distribution of aciclovir at steady state is 70% of the total bodyweight. Plasma protein binding is about 15% and plasma half-life is 2.5-3.0 h. Approximately 70% of aciclovir is excreted unchanged in the urine.

Figure 7
Mechanism of action of aciclovir



The dose should be reduced in renal failure. There is only one important metabolite: 9-carboxy-methoxymethylguanine without antiviral activity. About 10% of the dose is excreted in the urine as this metabolite [18].

Repeated intravenous doses of aciclovir 5 mg/kg every 8 h resulted in a steady-state mean peak plasma aciclovir concentration of 9.7 mg/l. Oral administration of 200 mg aciclovir every 4 h resulted in concentrations of 0.5 mg/l [18].

Aciclovir is the drug of choice for the treatment of herpes simplex virus. It is an effective treatment for initial and recurrent episodes of genital herpes (HSV-2). Aciclovir does not cure genital herpes, but diminishes the symptoms. Oral as well as intravenous treatment of initial genital herpes with aciclovir reduced the duration of viral shedding, new laesion formation, and the duration of both local and systemic symptoms; the overall mean healing times were halved [19].

For initial treatment, oral therapy, with a dosage of 200 mg 5 times a day for 10 days is recommended. In severe cases aciclovir 5 mg/kg every 8 h intravenously is an effective therapy. For external laesions 5% topical aciclovir (in polyethylene glycol) may be beneficial [19]. The benefits of aciclovir in recurrences of genital herpes simplex virus are less pronounced. It is important that therapy is started early. Prevention of new laesion formation by early aciclovir therapy has been reported [19]. Suppressive therapy has to be considered in patients with severe and frequent (six or more) recurrences a year. With continuous oral therapy (400 mg twice daily) the genital herpes recurrence rate is reduced by about 85% [20].

In 99% of the cases orofacial herpes is caused by HSV-1. The virus remains latent in neural ganglia following a primary infection and recurs more or less frequently. In immuno-competent patients HSV-1 can be treated with topical aciclovir (5% aciclovir cream). In case of severe recurrences oral drug therapy may be worthwhile (200 mg 5 times a day). Herpes simplex keratoconjunctivitis can effectively be treated with 3% aciclovir ointment [15].

Reactivation of HSV-1 infection can be effectively suppressed by oral (800 mg a day) and intravenous (5 $mg \cdot kg^{-1} \cdot d^{-1}$) aciclovir in immunocompromised patients [9]. Herpes encephalitis can be treated with aciclovir (10 mg/kg intravenously every 8 h for 10 days). It is superior to vidarabine (15 $mg \cdot kg^{-1} \cdot d^{-1}$ for 10 days). The mortality was 19% in the aciclovir-treated group versus 50% in the vidarabine group [21]. In the case of neonatal herpes intravenous aciclovir can be used (10-15 mg/kg thrice daily intravenously for 10 days). This therapy is equally effective as vidarabine 30 $mg \cdot kg^{-1} \cdot d^{-1}$ for 10 days but the mortality rate is still 20% [22].

The primary varicella zoster virus infection is chickenpox. When a varicella zoster virus infection is reactivated it expresses itself as herpes zoster. Especially in neonates and immunocompromised patients varicella zoster virus causes considerable morbidity and mortality. Immuno-compromised hosts can be effectively

treated intravenously with 500 mg/m^2 thrice daily for 7 days. In otherwise healthy persons often oral therapy with aciclovir (800 mg 5 times a day) for 7 days will be sufficient [2 15 23 24]. 4 g A day is a rather high dose, which is connected with the poor absorption of aciclovir.

Adverse effects of aciclovir are rare. The major problem has been the deposition of aciclovir in the kidneys following rapid bolus infusion. This problem can be avoided by sufficient hydration of the patient (1 l of fluid for each g of intravenously administered drug) [15]. Gastro-intestinal side-effects have been reported: nausea, vomiting and abdominal pain, but these effects are not severe. Very occasionally central nervous system toxicity has been noted [2 9 15]. Intravenous aciclovir therapy has been associated with inflammation and phlebitis at the injection site (15%) [18].

For a couple of years efforts have been made to develop a better absorbable aciclovir analogue. Desciclovir (BW A515U, 6-deoxyaciclovir) is an example of such a drug. Desciclovir has no detectable antiviral activity *in vitro*. However, it is well-absorbed (75%) from the gastro-intestinal tract and rapidly converted by xanthine oxidase to aciclovir. The half-life of desciclovir was 0.85 ± 0.16 h. Desciclovir orally 250 mg thrice daily gave aciclovir plasma levels comparable to a dose of 2.5 mg/kg aciclovir intravenously thrice daily [25]. Almost two-thirds of the absorbed dose was recovered in the urine as aciclovir. No serious or consistent adverse effects were noted. This may give us the possibility to treat serious infections with oral desciclovir instead of parenteral aciclovir, but this needs to be confirmed in clinical trials.

Ganciclovir

The new antiviral agent ganciclovir is closely related to aciclovir. Both are guanosine derivatives (Fig. 6). The mechanism of action resembles the mechanism of aciclovir. Ganciclovir is phosphorylated to ganciclovir monophosphate. The cellular enzymes responsible for this phosphorylation of ganciclovir are not known. As far as we know cytomegalovirus is not capable of encoding a virus-specified thymidine kinase. Probably the phosphorylation to the monophosphate is caused by a virus-induced cellular enzyme (deoxyguanosine kinase). Cellular kinases are able to phosphorylate the monophosphate to ganciclovir diphosphate and ganciclovir triphosphate. Firstly, ganciclovir triphosphate, competitively inhibits the incorporation of deoxyguanosine triphosphate into DNA by DNA polymerase. Secondly, the incorporation of ganciclovir triphosphate in viral DNA causes ending or strong limitation of viral DNA elongation. Ganciclovir is preferentially phosphorylated in infected cells. However, the uninfected cell is capable of producing ganciclovir triphosphate in low levels. Accumulation of ganciclovir triphosphate may result in myelosuppression [26].

Ganciclovir *in vitro* is a strong inhibitor of all herpes viruses, including Epstein-Barr virus and cytomegalovirus. The ID50 for cytomegalovirus strains varies from 0.8-11 μmol/l (median 2.15 μmol/l). For HSV-1 the ID50 ranged between 0.2 and 3 μmol/l and for HSV-2 between 0.2 and 13.2 μmol/l. The ID50 for varicella zoster virus ranged between 4.4 and 8.8 μmol/l [26].

Recently, three cases of cytomegalovirus resistant to ganciclovir were described. The mechanism involved in the development of ganciclovir-resistant cytomegalovirus is not clear. So, ganciclovir has to be used carefully and only in patients in whom cytomegalovirus disease is potentially fatal or in whom loss of vision is likely to occur [27].

Ganciclovir exhibits bi-exponential decay, with a terminal half-life of approximately 2.5 h and a relatively large volume of distribution (V= 32.8 l/1.73 m^2). Oral bioavailability of ganciclovir is poor (3%). At steady state the mean maximal plasma concentration is 32.6 μmol/l (dose 5 mg·kg^{-1} twice daily). No ganciclovir metabolite has been detected. Urinary recovery averages more than 90% in 24 h. Renal insufficiency requires a lower dose according to the creatinine clearance [26].

Cytomegalovirus disease is a major problem in immuno-compromised patients, caused by immuno-suppressive drugs or acquired from an underlying disease, such as AIDS. Serological evidence of cytomegalovirus infections are present in over 90% of patients with AIDS. The mortality and morbidity of cytomegalovirus disease is considerable. Until recently no therapy was available for the treatment of cytomegalovirus infections and ganciclovir is the first antiviral agent with antiviral properties against cytomegalovirus. Because of its toxicity (myelosuppression), ganciclovir is only used in the treatment of cytomegalovirus infections. In the case of herpes simplex virus and varicella zoster virus infections aciclovir is preferable [26]. In several clinical investigations ganciclovir appears to be a promising antiviral drug for cytomegalovirus infection.

Collecting the data on 314 immuno-compromised patients with serious cytomegalovirus infection, the intravenous administration of ganciclovir shows good clinical response: 84% of cytomegalovirus retinitis, 83% of gastro-intestinal cytomegalovirus infection and 26% of cytomegalovirus pneumonia improved [28]. After discontinuation of ganciclovir relapse of cytomegalovirus disease occurs and a life-long maintenance treatment is necessary.

The initial dose is 5 mg/kg twice daily in a 1-h infusion for 14 to 21 days. Maintenance therapy requires doses of 6 mg/kg a day for 5 days a week or 5 mg/kg a day for 7 days a week by intravenous infusion. The most important side-effects include neutropenia (42%) and thrombocytopenia (19%), central nervous system effects, like headache, psychosis, confusion and depersonification (18%) and gastro-intestinal problems, like nausea, vomiting and diarrhoea. Severity of side-effects is dose-dependent and neutropenia seems to be the dose-limiting adverse effect. However, it is not known whether neutropenia is dose-related or idiosyncratic. Because of this side-effect it is sometimes necessary to stop infusion therapy. For the treatment of cytomegalovirus

retinitis intravitreal injection appears to be a good alternative [29].

Foscarnet

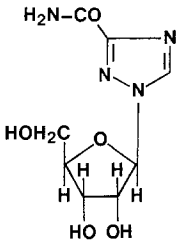


Figure 8
Structure of foscarnet

Foscarnet (phosphonoformate) has been known for over a decade as an antiviral compound. It is a pyrophosphate analogue (Fig. 8). How foscarnet exactly acts is unknown. It inhibits reversibly and competitively viral DNA polymerase of all herpes viruses and the RNA polymerase of influenza viruses probably by binding with a pyrophosphate binding site on the polymerases. Foscarnet also inhibits retroviruses and HIV by non-competitive inhibition of reverse transcriptase. Because foscarnet is not a nucleoside analogue, no (viral) thymidine kinase is involved in the mechanism of action [30].

In vitro foscarnet exerts activity against all human herpes viruses and some retroviruses (*e.g.* HIV). Most of the clinical cytomegalovirus cultures are sensitive to 200 μmol/l foscarnet.

Foscarnet is a virustatic agent and effective treatment needs continuous administration. The oral bioavailability varies from 12-22% and continuous infusion is necessary. Another problem of foscarnet is its incorporation in the bone matrix. About 10 to 28% of the cumulative dose may have been deposited in bone 2 days after infusion [31]. Plasma protein binding is 17%. Foscarnet is mainly eliminated unchanged by the kidneys and nephrotoxicity has been described. Because of these inconveniences the use of foscarnet is limited to cytomegalovirus retinitis in patients with AIDS as an efficacious alternative to ganciclovir, but direct comparative studies are needed [32]. Recently, it has been described that foscarnet appears to be an effective, relatively nontoxic drug for cytomegalovirus retinitis by intermittent intravenous administration. 10 Patients received 60 mg/kg bodyweight every 8 h for 14 days and 9 of them had stabilized or improved. Maintenance therapy consisted of 60 mg/kg as a single daily infusion for 5 days a week [33]. This could be an interesting new application of foscarnet, but additional studies are needed.

In the treatment of herpes simplex and varicella zoster infections aciclovir remains the treatment of choice.

Ribavirin

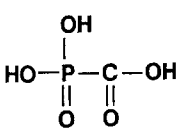


Figure 9
Structure of ribavirin

Ribavirin is a synthetic nucleoside that structurally resembles a pyrimidine nucleoside (Fig. 9). It is rapidly transported into cells and metabolized by cellular enzymes to monophosphate, diphosphate and triphosphate derivatives, which then inhibit viral nucleic acid synthesis. The mechanism of action is not yet fully elucidated. Ribavirin monophosphate is able to interfere with the formation of guanosine monophosphate, so viral enzyme systems dependent on guanosine are therefore unable to complete their own transcription. Probably ribavirin also interferes with the capping and translation of mRNAs [34].

Ribavirin exhibits a virustatic effect against a variety of both RNA and DNA viruses, such as influenza A and B, respiratory syncytical virus, measles, *para*-influenza, mumps, reoviruses, certain coxsackie strains, Venezuelan equine encephalitis, herpes simplex types 1 and 2, Lassa fever, hepatitis A, and human immuno-deficiency virus. With this spectrum ribavirin can be considered a broad-spectrum antiviral agent. However, results from clinical trials are conflicting and clinical uses are limited [35]. The *in vitro* minimal inhibitory concentration against respiratory syncytial virus is 3-32 mg/l [34].

Ribavirin may be administered orally, intravenously or by small-particle aerosol. Oral administration (3 mg/kg) to healthy subjects results in peak plasma concentrations of 1-2 mg/l after 1 to 1.5 h. The elimination half-life is about 24 h. Intravenous administration of 1,000 g followed by 4 g/day in 4 doses for 4 days and for the next 6 days 1,500 mg/d in 3 divided doses leads to plasma levels of about 20 mg/l. Renal secretion is the main route of elimination (32-53% in 72 to 80 h). A small portion of the drug is faecally excreted (15%). The bioavailability of ribavirin aerosol is unknown [9 34].

At this moment ribavirin is only indicated for the treatment of lower respiratory tract infections due to respiratory syncytial virus in hospitalized neonates and young children with severe underlying diseases like pre-existent cardiovascular and pulmonary disease or immunodeficiencies. Respiratory syncytical virus infection has to be confirmed. Ribavirin is administered by small-particle aerosols. Treatment has to be started within 3 days after the infection occurred and has to be continued for a period of 3 to 7 days, 18 to 20 h a day. The suggested dose for children is about 1.4 $mg \cdot kg^{-1} \cdot h^{-1}$ and for adults 0.82 $mg \cdot kg^{-1} \cdot h^{-1}$. This route of administration has as the major advantage that very high levels of ribavirin are reached at the site of viral replication, with only little systemic absorption. No side-effects were noted [34]. However, there is doubt about the efficacy of ribavirin in the treatment of respiratory syncytical virus. Only a limited number of publications are available about the benefits of ribavirin in the treatment of respiratory syncytical virus. There is a lot of criticism about the methods of investigation [35]. Beside the doubts about the efficacy, ribavirin therapy is very expensive. Ribavirin is reported to be effective in patients with Lassa fever. Intravenous therapy (1 g intravenously 4 times a day for 4 days followed by 0.5 g every 8 h for 6 days) reduced the mortality rate from 76 to 32%. Oral ribavirin 333 mg 3 times a day for 10 days reduced it from 76 to 30% [36]. The most important side-effect of intravenous therapy is anaemia. Increases in inconjugated serum bilirubin and decreases in reticulocyte count have been described [9]. The results of the trials until this moment do not justify extensive use of this drug.

Interferons

Interferons are glycoproteins produced by each species of animals. They are a part of the natural host defences and have a broad-spectrum antiviral activity. Interferons can be divided into three main types: interferon-α, interferon-β and interferon-γ. Interferon-α and interferon-β are in-

duced by stimuli like viruses, bacteria and double-stranded RNA and are produced by leukocytes and fibroblasts, respectively. Interferon-γ is produced mainly by lymphocytes in response to mitogenic or antigenic stimuli. After release, interferons bind to cell surface receptors, whereby interferon-α and -β have a common cellular receptor and exert probably a similar mechanism of action. Interferon-γ has a separate receptor.

Interferons can inhibit many stages of the virus life-cycle by induction of cellular enzymes in cells exposed to interferon. As a result breakdown of viral RNAs and inhibition of viral protein formation occurs. But there may be more mechanisms of action. For retroviruses it has been described that they inhibit assembly and release of viral particles [2 37 38].

Interferon-α and interferon-β have activity against a broad range of viruses. Interferon-γ has also an antiviral activity. Interferon-α is the one mainly used as antiviral agent.

Interferon-α can be administered intravenously, intramuscularly, intrathecally, intralaesionally, as a nasal spray, or as topical cream. Peak serum levels are about 15-50 IU/ml for a $3 \cdot 10^6$ IU dose intramuscularly. Serum half-life is 6-8 h. Metabolization occurs mainly in the kidney although uremic patients do not show accumulation [37].

Interferons have a limited clinical role as antiviral agent. Intranasal interferon-α is used in the prophylaxis of rhinovirus colds. Given by spray or on cotton pledgets it has been shown to protect for about 80% against the transmission of rhinovirus to family members, but it was not active against colds caused by other viruses [39]. Unfortunately, prolonged prophylactic use is associated with nasal discomfort, dryness of the mucosa and the discharge of blood-stained mucus (14%).

Under investigation is the treatment of papillomavirus-related conditions, like condylomatosis or juvenile papillomatosis, but it is yet to be established. Parenteral interferon-α may have a role in chronic viral diseases like non-A, non-B hepatitis and chronic hepatitis B, but the results of small-scale studies need to be confirmed [2 38]. *In vitro* interferon-α has a weak activity against HIV, but synergy with zidovudine, foscarnet and dideoxycytidine has been described. In clinical trials combination therapy of interferon-α with zidovudine is used. Because of the side-effects (bone-marrow toxicity), high-dose interferon-α (>9 MU) is incompatible with full dose zidovudine. Trials with the combination against HIV are ongoing [40].

Side-effects are a influenza-like syndrome, persistent fatigue, peripheral neuropathy and bone-marrow suppression. Persistent fatigue is the most important dose-limiting side-effect. Bone-marrow suppression (mild neutropenia, mild anaemia and thrombocytopenia) are reversible.

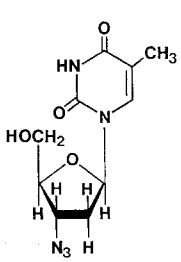


Figure 10
Structure of zidovudine

Zidovudine

Zidovudine (azidothymidine, AZT) is a thymidine analogue (Fig. 10). It is active against HIV. As for the other nucleoside analogues, zidovudine is intracellularly converted to zidovudine triphosphate by thymidine kinase, thymidilate kinase and other cellular enzymes which phosphorylate thymidine. These enzymes are cellular enzymes. HIV does not code for its own kinases nor stimulates HIV cellular kinases. Zidovudine has no effect on extracellular virions. Zidovudine triphosphate inhibits HIV multiplication by inhibition of viral reverse transcriptase and termination of the viral DNA chain [41]. *In vitro* zidovudine has been found to be active at concentrations of approximately 1 μmol/l.

Zidovudine can be administered intravenously or orally. The bioavailability of oral zidovudine is approximately 60% and peak plasma concentrations are reached 0.5 to 1.5 h after administration. The peak plasma concentration varies approximately in a linear fashion with the dosage. An oral dose of 200 mg results in a peak plasma concentration of about 2.4 μmol/l. Zidovudine is metabolized in the liver by glucuronidation, which product is excreted in the urine. The elimination half-life is about 1 to 1.5 h [41].

Zidovudine is licensed for the treatment of patients with severe manifestations of infection with AIDS or AIDS-related complex. However, after a couple of years experience with zidovudine it seems that the benefits are limited to the first 6 months of therapy, where the maximum effect is reached. After 6 months the clinical efficacy strongly decreases. One of the causes of declining effectivity could be haematological toxicity of the drug, which led to interruption of treatment [42]. The other possibility is the appearance of mutants of HIV with decreased sensitivity to zidovudine [43].

Besides the licensed indication zidovudine is under investigation in treating patients with lymphadenopathy, central nervous system disease, symptoms of HIV infection and in treating seropositive asymptomatic patients. Prophylactic use of health-care workers after needle-prick injury is also under investigation. Recently it was described that after injecting a minimal volume of seropositive blood, fast onset of zidovudine therapy did not prevent HIV-1 infection [44]. The recommended dose of zidovudine is 200 mg every 4 h, but there are many experimental variations on this dose and interval. Optimum dosage in children and in elderly patients with renal or hepatic function insufficiency are not known.

The main adverse effects are anaemia, neutropenia and leukopenia indicating bone-marrow suppression (45%). At the start of therapy gastrointestinal disturbances, headache and myalgia frequently occur, but these diminish or disappear with continued administration. Especially the haematological complications lead to dose reduction or transient interruption of zidovudine treatment. Maybe combination therapy of low-dose zidovudine with other antiviral drugs will become the future strategy.

Future directions in antiviral therapy: highlights

Most efforts in developing new antiviral drugs are made in the development of anti-HIV drugs. On theoretical grounds there are many modes of

intervention as described earlier. Agents that block attachment to the cell inhibit infection of new cells, but do not affect chronically infected cells. By blocking transcription of the retroviral genome to HIV-specific messenger RNAs it is theoretically possible to stop new infections by inhibiting late steps in virus replication. This leads to a considerable list of 'hypothetically' active agents. From these agents only a few have already reached the clinic, while others are in the laboratory stage [40]. We will describe here the most promising agents for the near future.

Soluble CD4

The first important step in HIV infection is attachment to the cell. A specific cellular receptor on the CD4 molecule is the binding place. Probably the HIV envelope protein gp120 is involved in this interaction. So, blocking the receptor on the CD4 molecule or blocking the viral protein gp120 is a possibility to prevent cellular viral infection. Modern biotechnology allows production of recombinant-soluble-CD4 (rsCD4).

Soluble CD4 can be administered by intramuscular or subcutaneous injection. The bioavailability is 51% and 45%, respectively. The rsCD4 is rapidly cleared. After intravenous administration the serum half-life is approximately 45 min. After intramuscular injection peak serum levels are reached after 4 to 6 hours, suggesting that the intramuscular compartment serves as a reservoir [45 46].

CD4 is under clinical investigation now in phase I-II studies. In a small-scale escalating dosage trial 25 patients received 27 courses of CD4 intramuscularly or by intravenous infusion. Dosages used were 0.9, 3, 9 or 30 mg a day. The drug was well-tolerated and provided preliminary evidence of antiviral activity *in vivo* [45]. In another study 42 subjects received doses of up to 300 μg/kg bodyweight per day intravenously. It was concluded that rCD4 was safe and well-tolerated [46]. However, much more data about long-term toxicity and efficacy are needed.

Dideoxyadenosine and dideoxyinosine

Closely related to zidovudine are dideoxyadenosine and dideoxyinosine. Both are purin analogues (Fig. 11). Dideoxyadenosine is extra- and intracellularly converted to dideoxyinosine. In human cells, dideoxyinosine is metabolized to its active moiety, dideoxyadenosine triphosphate. Dideoxyadenosine triphosphate acts as a substrate and inhibitor of HIV reverse transcriptase, blocking the synthesis of a DNA copy of the viral genome. In combination with antacids the bioavailability of dideoxyinosine is 40%. (Dideoxyinosine is acid labile so combination with antacids is necessary). Intravenous doses of 1.6 mg/kg and oral doses of 3.2 mg/kg showed favourable effects. Dideoxyadenosine triphosphate has a long half-life of over 12 h in cells exposed to dideoxyinosine, so 12 h dosing seems sufficient [47]. Only limited clinical experience is available, but on the ground of these data the Food and Drug Administration have decided to make dideoxyinosine available for 'compassionate' use. Daily doses of dideoxyinosine below 1.5 g were well-tolerated in the short term. The CD4 lymphocyte count increased and p24 antigen levels decreased, suggesting antiviral efficacy [47].

Main side-effects were peripheral neuropathy, hyperuricemia, skin rash (3.8%), increased liver enzyme concentration, severe pancreatitis (3.8%) and seizures (7.6%).

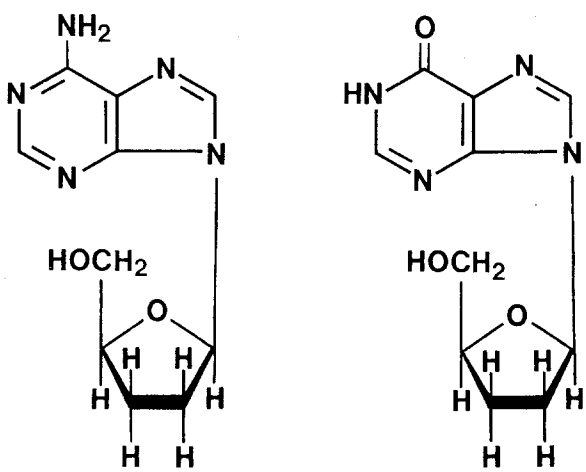


Figure 11
Structure of dideoxyadenosine (left) and dideoxyinosine (right)

Viral resistance

As mentioned before, there are an increasing number of reports dealing with viral resistance. It is not always clear what kind of mechanism underlies this resistance. Rimantadine resistance of influenza A virus (H3N2 subtype) was correlated with the presence of point mutations in the RNA sequence coding for a specific protein (M2 proteins), which results in resistance to rimantadine and amantadine [10]. There are at least 3 mechanisms for aciclovir resistance. First of all and most important is the selection of thymidine kinase-resistant mutants of virus. Other possibilities consist in alteration of the substrate specificity of the viral thymidine kinase and in the selection of viruses with an altered DNA polymerase [15 16]. Because of structural resemblance between aciclovir and ganciclovir it is expected that ganciclovir resistance would be of the same type as aciclovir resistance, but the exact mechanism is not yet elucidated [16 27]. It is not known whether the resistant strains are as virulent as the original virus and therefore clinical significance of viral resistance has to be determined in more extensive studies. It is clear, however, that antiviral drugs must be used carefully, like antibiotics.

Conclusions

Despite much research, progress in antiviral therapy has been slow. An important problem is the close connection between virus and host cell, which makes it difficult to distinguish between them. Nevertheless, we are able to treat viral infections to a certain extent and some new developments are promising (Table 2).

The first antiviral drug (idoxuridine) was too toxic for systemic use, but is still available for local treatment of herpes simplex virus. Aciclovir is useful for the treatment of many herpes virus infections (HSV-1, HSV-2, varicella zoster virus)

orally, systemically and locally, while vidarabine could be an alternative in case of aciclovir resistance. Amantadine (and rimantadine) is used in the prophylaxis and the treatment of influenza A. Two new drugs, ganciclovir and foscarnet have shown benefits in the treatment of cytomegalovirus infections. Ribavirin is under discussion.

Aerosolized ribavirin is effective for severe respiratory syncytial virus. Intravenous and oral therapy is reported to be beneficial for Lassa fever. Interferons play only a limited role as antiviral agent until now, but trials in combination therapy are ongoing. Against HIV zidovudine is extensively used.

All antiviral drugs mentioned are only virustatic. None of them is able to kill the virus, so recurrences always occur and ask for maintenance therapy. There is a need for drugs that can kill a virus.

Two major pathways in developing new drugs can be distinguished. Firstly, preventing the virus from infecting the cell. An example of such an approach is soluble CD4, binding to HIV and preventing the attachment of HIV to a host cell. Secondly, to prevent a virus in a host cell to multiply itself. One of the new drugs dealing with that approach is dideoxyinosine. Both drugs are under clinical investigation now.

Another problem we are facing is an increasing

Table 2
Therapeutic use of antiviral drugs

Antiviral drug	Virus and indication	Treatment schedule
Amantadine or rimantadine	influenza A	
	– prophylaxis	100-200 mg/day orally for 5-7 days
	– treatment	
Tromantadine	herpes simplex	1% (eye) ointment until 10 days after healing
Idoxuridine	herpes simplex	0.2% cream or ointment for 4 days
		0.1% eye drops for 4 days
	varicella zoster	0.2-0.5% eye ointment for 4 days
		5-10% in dimethylsulfoxide
Trifluridine	herpes simplex	1% eye drops
		2% eye ointment until 8 days after healing
Vidarabine	herpes simplex	3% eye ointment for 3-5 days
	varicella zoster	5% eye drops
		15-30 $mg \cdot kg^{-1} \cdot d^{-1}$ intravenously for 10 days
Aciclovir	herpes simplex	
	– keratitis	3% aciclovir eye ointment
	– herpes labialis	5% aciclovir cream
	– genital herpes	
	– primary and recurrent	1,000 mg/day orally for 5-10 days
	– prophylaxis	400-1,000 mg/day orally
	encephalitis	10 mg/kg thrice daily intravenously for 10 days
	neonatal herpes simplex virus	10-15 mg/kg thrice daily for 10 days
	immuno-compromised host	250 mg/m^2 thrice daily intravenously for 7 days or
	– treatment	1,000 mg/day orally for 10 days
	– prophylaxis	250 mg/m^2 thrice daily intravenously or 800 mg/day orally
	varicella zoster eye infections	3% eye ointment until 5 days after healing
	shingles	5-10 mg/kg intravenously for 5 days or 4,000 mg/day orally for 7 days
	immuno-compromised host	500 mg/m^2 thrice daily intravenously for 7 days
Ganciclovir	cytomegalovirus in immuno-compromised host	initial: 5 mg/kg twice daily for 14 days maintenance: 5 $mg \cdot kg^{-1} \cdot d^{-1}$ intravenously for 7 days or 7 $mg \cdot kg^{-1} \cdot d^{-1}$ intravenously for 5 days
Foscarnet	cytomegalovirus in immuno-compromised host	initial: 20 mg/kg in 30 min maintenance: 230 mg intermittent 60 mg/kg thrice daily for 5 days
Ribavirin	respiratory syncytical virus	aerosolized
	– adults	0.82 $mg \cdot kg^{-1} \cdot h^{-1}$, 12-24 h, 3-7 days
	– neonates	1.4 $mg \cdot kg^{-1} \cdot h^{-1}$, 12-24 h, 3-7 days
	Lassa fever	4 g/day for 4 days, 1.5 g/day for 6 days
Interferons	rhinovirus	intranasal spray
		$5 \cdot 10^6$ IU/d for 7 days
Zidovudine	human immuno-deficiency virus	200 mg 6 times daily, life-long investigational other dosages regimens

number of virus strains, resistant to the usual therapies. We may be obliged to use combination therapy in the future, but trials on this subject are needed.

Some fluoroquinolones, like ofloxacin and ciprofloxacin, show some degree of antiviral activity. Over 20,000 fluoroquinolone derivatives have been synthesized. When these drugs are tested for antiviral activity, it seems quite likely that potentially useful antiviral agents may be developed from these antibacterial agents. Their mechanism of action against viruses is as yet unknown.

It may be clear that a lot of work still needs to be done before we have safe and effective antiviral drugs.

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