Review

Achievements and challenges in antiviral drug discovery

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The last 40 years have seen the development of several antiviral drugs with therapeutic value in treating life-threatening or debilitating diseases such as those caused by HIV, hepatitis B virus, herpesviruses (such as herpes simplex virus and varicella zoster virus) and influenza virus. These relatively recent advances have been due to technical breakthroughs in the cultivation of viruses in the laboratory, identification of viral enzymes and, more recently, their molecular biology. We describe here the antecedence of several of the existing antivirals and their strengths and weaknesses. We indicate where the major challenges lie for future improvements of current therapies and possible new indications, such as hepatitis C virus and papillomavirus. We also describe how current antiviral therapies are restricted to a rather limited number of viral diseases of sufficient interest to the pharmaceutical industry. Finally we describe the potential threat of emerging viruses and bioweapons and the challenges that they present to therapy.

Keywords: antiviral, HIV, herpesvirus, hepatitis, drug discovery

Introduction

It is now 40 years since the New York Academy of Science sponsored the *1st Conference on Antiviral Substances*. At that time, viral replication was thought to be carried out by cellular enzymes and the possibility of a selective inhibition of viral replication looked bleak. In 1967, Kates and McAuslan described the first viral enzyme, pox virus DNA-dependent RNA polymerase (Kates & McAuslan, 1967), and hence the first mechanistic basis for selective antiviral drugs, which was soon to be followed by many other viral enzymes. At the *2nd Conference on Antiviral Substances* in 1969, the progress in this area was evident by three seminal announcements. Firstly, iododeoxyuridine, described earlier by Prusoff (Prusoff 1963), had been shown to be active against herpes simplex. Secondly, amantadine had been shown not only to inhibit influenza virus, but also to cause resistance development (Oxford *et al.*, 1970), later proposed to be a hallmark of selective antiviral effect (Hermann & Hermann 1977). Finally, interferon (IFN) and its inducers were extensively discussed as potential antiviral drugs against several different viral infections.

The field of antiviral drugs grew rapidly in the herpes area with the development of aciclovir (Schaffer *et al.*, 1978) and other inhibitors. However, a major stimulus to the development of antiviral drugs was the emergence of HIV/AIDS in 1983 which, in a short time, resulted in a large number of anti-HIV drugs targeting several viral enzymes. The maturity of the field today is illustrated by a recent review of the history of antiviral drugs (Field & De Clercq, 2004).

Attempts to develop drugs against influenza have been ongoing for decades and have resulted in potent and safe drugs, but with only limited use and commercial value. Similarly, effective inhibitors of rhinovirus have been developed but their progress has stalled, in part due to the limited clinical effect, which has led to a lack of a clear commercial case. Development of drugs against hepatitis B virus (HBV) has been successful and there is a large effort to discover drugs against hepatitis C virus (HCV). The medical need for therapy against human papilloma virus (HPV) infections has been hampered by the presence of only one, somewhat unattractive, viral enzyme, a DNA helicase (which is notoriously difficult to express in recombinant systems and to develop assays), as a potential target. Interestingly, new therapies for HPV are focused largely upon immunomodulatory approaches including therapeutic vaccines. Very recently, bioterrorism has created an interest in therapies against viral diseases traditionally of little economical interest to the large pharmaceutical companies.

In this review, we will discuss the development of antiviral drugs in the perspective of medical need, technical possibilities and economic restrictions.

Rationale for development of antiviral drugs

The high cost of developing drugs has limited the number of viral diseases of sufficient market size to a relatively short list. The fact that antiviral drugs are likely to be highly specific for one single infectious agent dictates that accurate diagnosis of an infection needs be made before therapy; this also significantly limits the number of diseases of commercial interest. For this reason, and others, a severe disease with a long duration and high incidence is the ideal target. The presence of effective vaccines, and the degree to which they are used, are, of course, also important factors when considering the need for drug development. Even when an effective vaccine exists, as is the case for HBV, the presence of patients who are already infected may provide a sufficiently large market for chemotherapy. These considerations are summarized in a simplified form in Table 1. It is not surprising that the major industrial effort today is directed to therapies for HIV and HCV. Unfortunately, whatever the clinical severity, research on inhibitors of less common viral infections is unlikely to result in development of drugs by the pharmaceutical industry. However, 30 years ago, the list would not have included HIV and HCV – new infections will spread and old diseases may be found to have a viral basis, thus making the list of commercial targets for antiviral therapy longer. Bioterrorism could, unfortunately, also augment the list.

Herpesviruses

The drugs licensed for the treatment of herpesvirus infections are shown in Table 2. Apart from the antisense compound vitrovene all the other compounds are DNA polymerase inhibitors of which the nucleoside analogues require phosphorylation by viral kinases.

Herpes simplex virus type 1 and 2 (HSV-1 and 2) infections

Labial herpes (cold sores mainly caused by HSV-1) and genital herpes (mainly caused by HSV-2) are recurrent diseases afflicting a large number of individuals worldwide.

Table 1. Rationale for the pharmaceutical industry to develop antiviral drugs

Virus	Vaccine available	High incidence	Severe	Easy diagnosis	Long duration	Viral enzyme
Rhino	-	+	–	–	–	+
Influenza	+	+	–/+	–/+	–	+
RSV	-	–/+	–/+	–/+	–	+
HCV	-	+	+	+	+	+
HIV	-	+	+	+	+	+
HBV	+/-	+	+	+	+	+
Papilloma	-	+	–/+	+	+	–/+
Herpes	+/-	+	–/+	+	–/+	+

RSV, respiratory syncytical virus; HCV, hepatitis C virus; HBV, hepatitis B virus.

Table 2. Antiviral drugs against herpesvirus infections

Infectious agent	Generic name of compound	Trademark	Type of inhibitor
HSV, VZV	Acyclovir (ACV)	Zovirax®	NA polymerase inhibitor
HSV, VZV, HCMV	Valaciclovir (VCV)	Valtrex®	NA polymerase inhibitor
HSV	Penciclovir (PCV)	Vectavir®	NA polymerase inhibitor
HSV, VZV	Famciclovir (FCV)	Famvir®	NA polymerase inhibitor
HSV, HCMV	Foscarnet (PFA)	Foscavir®	Pyrophosphate analogue, polymerase inhibitor
HSV	Idoxuridine (IDU)	Herpid®	NA polymerase inhibitor
HSV	n-Docosanol	Abreva®	Detergent
HCMV	Ganciclovir (GCV)	Cymmevene®	NA polymerase inhibitor
HCMV	Cidofovir (HPMPC)	Vistidine®	NA polymerase inhibitor
HCMV	Fomivirsen	Vitrovene®	Antisense

HCMV, human cytomegalovirus; HSV, herpes simplex virus; VZV, varicella zoster virus; NA, nucleoside analogue.

HSV-1 and 2 can also cause eye disease, encephalitis and generalized infections in immunodeficient patients and newborns. To date, vaccination against HSV has not been successful but chemotherapy and chemoprophylaxis are available. However, therapy against recurrent episodes of labial and genital herpes has a rather modest effect in shortening the disease episode.

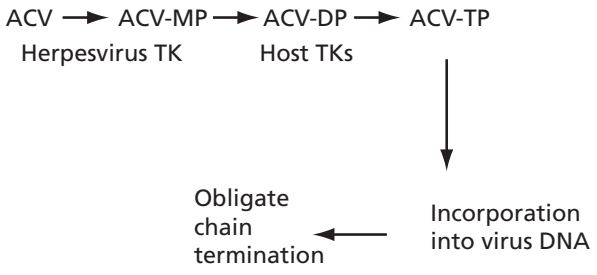
The development of aciclovir was a breakthrough in the antiviral field (Elion *et al.*, 1977). In the case of HSV, varicella zoster virus (VZV) and Epstein-Barr virus (EBV), aciclovir acts through the function of two herpesvirus enzymes. The first herpesvirus enzyme is the thymidine kinase (TK), which phophorylates aciclovir to the mono- and, in the case of HSV-1, then the diphosphate. The triphosphate is subsequently formed by the action of cellular enzymes. The second enzyme is the viral-encoded DNA polymerase, which is the target inhibited by aciclovir triphosphate by a mechanism known as obligate chain termination (see Figure 1). Whilst there are other mechanisms by which aciclovir triphosphate may inhibit the HSV DNA polymerase (such as competitive inhibition of the natural guanosine triphosphates for the DNA polymerase), it is clear that, in the clinical setting, the levels of aciclovir triphosphate generated are sufficient only for chain termination (Reardon, 1989). In the case of the nucleoside analogues penciclovir and ganciclovir, the inhibition of the HSV polymerase is not 'obligate' chain termination as a pseudo 3′ hydroxyl is available on the molecule. It appears that, in this case, termination occurs several nucleosides after the addition of the nucleoside analogue, suggesting that the incorporation somehow alters the conformation of the replication complex and prevents extension of the DNA chain (Reardon, 1989; Earnshaw *et al.*, 1992).

Whilst this elegant and highly selective mechanism of action explains the rationale for activity of aciclovir against most herpesviruses, its activity against human cytomegalovirus (HCMV) was puzzling and enigmatic. As described below, aciclovir and its prodrug valaciclovir have been clinically demonstrated to be active against HCMV. Moreover, the nucleoside analogue ganciclovir, which has greater *in vitro* activity than aciclovir against HCMV, has been clearly shown to be useful in the treatment of HCMV disease. However, the mechanism of action of the nucleoside analogues for HCMV [and human herpesvirus 6 (HHV-6)] was not clear. Specifically, molecular analysis of HCMV by Chee *et al.* (1990) showed that HCMV lacked the TK found in other herpesviruses. This dichotomy was resolved when it was demonstrated that the locus for the genetic mutations for resistance against ganciclovir resided in a specific location on the HCMV genome, namely the protein kinase UL97 (Sullivan *et al.*, 1992). This was supported by biochemical evidence that the UL97 gene product could phosphorylate ganciclovir to its triphosphates *in vitro* (Littler *et al.*, 1992). These two findings have been confirmed by many subsequent studies on the genetic origins of resistance of clinical isolates of HCMV (Jabs *et al.*, 2001).

Both aciclovir, and its more bioavailable prodrug valaciclovir, have been shown to be effective in prophylaxis against episodes of genital herpes, thereby preventing painful episodes of genital sores (Griffiths, 2002). Penciclovir, and its prodrug famciclovir, which act by a mechanism similar to aciclovir (Hodge & Perkins, 1989), show benefit in prophylaxis against genital herpes. However, the therapeutic effect of topical aciclovir and penciclovir against recurring episodes of labial cold sores and recurring genital herpes is limited to about a 10% reduction in healing time, even if treatment is initiated during prodromal symptoms (Apoola & Ratcliffe, 2004). The reason for this may be the relatively short duration of viral replication and the fact that the major part of the symptoms found in a cold sore are caused by inflammation generated by the immune system. This is illustrated by the findings that topical aciclovir can be shown to reduce the healing time from 5 days to around 4.5 days (Spruance *et al.*, 2002). In contrast, a better effect is observed on primary herpes with a longer duration of HSV replication and a later immune reaction. However, this is a much smaller market than the recurrent labial and genital herpes and much more difficult to diagnose.

It seems likely that improvement of the therapeutic effect against recurrent episodes of herpes can only be achieved by a combination of inhibition of viral replication by, for example, aciclovir and concomitant reduction of the immune reaction by, for example, hydrocortisone. Recent results from clinical trials (Evans *et al.*, 2002) indicate that the combined effect of aciclovir and hydrocortisone gives a substantially better effect than the antiviral drug alone and that even prevention of ulcerative lesions is possible. Due to the immune suppressive nature of hydrocortisone, the

Figure 1. Mechanism of action of acyclovir



ACV-MP, acyclovir monophosphosphate; TK, thymidine kinase.

presence of an antiviral such as aciclovir is essential in this treatment.

The result of combination therapy against recurrent herpes is a reminder of the importance of relevant laboratory models in predicting clinical efficacy. Primary cutaneous HSV infections in rodents respond much better to therapy with aciclovir than recurrent episodes in humans. However, with a model in mice that simulates recurrent HSV infection (Harmenberg *et al.*, 2003), aciclovir and similar inhibitors show the same limited effect as in humans, while combinations of an antiviral drug and an immunomodulator (for example, aciclovir plus hydrocortisone) show substantial effects, later confirmed in humans (Evans *et al.* 2002).

Resistance development is a concern with most antiinfectious agents. However, in the case of aciclovir, HSV-1 and HSV-2 have not developed resistance in immunocompetent patients, despite many years of daily treatment (Christophers *et al.*, 1988). One explanation for this is that HSV mutants with resistance to aciclovir lack, in most cases, a functional viral TK; however, so-called TK-deficient viruses are unable to reactivate. Therefore, even if resistant virus should be selected during treatment it will not appear during the next episode (Jacobson *et al.* 1993).

VZV

Treatment of varicella (chickenpox) and shingles, both caused by VZV is possible with aciclovir, penciclovir and their prodrugs (Whitley & Gnann, 1999). The major market is for shingles, which is becoming more prevalent in the developed world due to an increasingly aging population. Vaccination against varicella has started in countries such as the USA and this might eventually change the market. However, although the live attenuated VZV vaccine is effective in preventing the clinical signs of chickenpox, it does not prevent the establishment of latency by the virus and there has been concern and speculation that the vaccine could possibly increase the prevalence of shingles. Repeated vaccination of the elderly to boost their immune system in order to decrease the likelihood of developing shingles is a possibility, but it is unlikely to be widely used considering the rather limited uptake of influenza vaccination in the elderly, in spite of the generally agreed clear beneficial effect.

A serious weakness of the present therapies against shingles is the inability to treat or reduce the incidence of postherpetic neuralgia (PHN), which is a serious and long-term consequence of the disease, more common with increasing age of the patient. In the laboratory and the clinic, aciclovir and penciclovir are less active against VZV than against HSV. Thus, there is a need for more potent inhibitors with improved pharmacokinetic properties which will ensure that the antiviral compound is delivered to the VZV-infected neural tissue and, therefore, reduce the likelihood and severity of PHN.

One such inhibitor is valomaciclovir, which is a prodrug of H2G, a nucleoside analogue similar to aciclovir but more potent against VZV in various *in vitro* and *in vivo* models (Soike *et al.*, 1993; Lowe *et al.*, 1995).

Cytomegalovirus (CMV)

CMV infection is a serious threat to immunodeficient patients, such as AIDS or transplant patients, causing a variety of conditions from retinitis to pneumonitis. Combination treatment against HIV has decreased AIDS and thus the incidence of CMV disease (Springer & Weinberg, 2004), but an increase in the number of transplant patients increases the need for CMV prophylaxis and therapy.

Ganciclovir and foscarnet have been used for treatment of CMV infections (Wagstaff & Bryson, 1994) and recent studies show valaciclovir to have a prophylactic effect against CMV disease in transplant patients (Griffiths, 1993, 2002).

In view of the efficacy of ganciclovir, aciclovir and valaciclovir against HCMV, and the relatively small number of patients, it is not clear that it would be commercially viable to develop further drugs against CMV although such compounds are being researched (De Clercq, 2003). Development of resistance to aciclovir and ganciclovir in CMV-infected patients may potentially change this situation but as yet there is no evidence that this is occurring to a significant extent, and such viruses are likely to be sensitive to foscarnet therapy (Gilbert *et al.*, 2002). Limitations of foscarnet therapy include the need for i.v. administration, risk for nephrotoxicity (which can be decreased by increasing fluid intake) and complex binding to calcium and magnesium (Palestine *et al.*, 1991).

Other herpesviruses

EBV is the cause of infectious mononucleosis, which is a significant medical problem for adolescents, largely from high socio-economic backgrounds, and for which there is presently a lack of antiviral therapy. *In vitro,* aciclovir and penciclovir are not very active against EBV although in clinical trials using intravenous aciclovir, EBV levels were decreased in saliva but not in blood (perhaps reflecting the fact that EBV in lymphoblastoid cells is largely latent). However, this is without any concomitant effect on clinical signs (Pagano *et al.*, 1983; Yao *et al.*, 1989). It must be emphasized that these preliminary studies were done with only short-term therapy and should encourage further, longer-term, antiviral therapies to be attempted. It will be of interest to see if a more potent inhibition of EBV, such as that by H2G (valomaciclovir) (Lowe *et al.*, 1995) could decrease symptoms in mononucleosis patients.

HHV types 6, 7 and 8 cause various human diseases (including, in the case of HHV-6, recurrent fitting in children) but probably not of a magnitude that would make the development of specific therapies economically feasible. However, as spin-off indications during development of new drugs against other herpesviruses it might also be possible to find effective treatments of these viruses. If HHV-6 was shown to cause multiple sclerosis as has been suggested (Sola *et al.*, 1993) or to transactivate HIV (Ensoli *et al.*, 1989), this would, of course, increase the interest in therapy of this virus.

Respiratory viruses

The drugs currently licensed for use against respiratory viruses are shown in Table 3.

Influenza

The clinical and social impact of influenza infections are poorly recognised in two ways. Firstly, in most years the number of deaths caused by influenza infections can be as high as 30 000 in the USA alone. However, during pandemics (thought to be caused by reassorted viruses generated in animals), which are known to have occurred regularly over the last century, the mortality figures can be startling. Indeed, the total number of deaths due to influenza in the most notorious pandemic of 1918 (so-called Spanish Flu) has been estimated to have killed >20 000 000 people worldwide. It is sobering to consider that this is more deaths than occurred during the hostilities of World War I which had come to a halt only a few months before (Oxford *et al.*, 2002). For these reasons, influenza virus disease has attracted considerable attention in the pharmaceutical industry. Despite the development of potent drugs directed against the viral M2 protein and the viral neuraminidase, they have not proven very profitable. It is illustrative for the field as a whole to explore the reasons for the commercial failure of these treatments.

In the 1960s, amantadine was found to be a useful drug against influenza, especially if used prophylactically (Dawkins *et al.*, 1968). Resistance development to amantadine was observed in the laboratory and the clinic, but this was not a major reason for the limited use in Western countries. A strong vaccine lobby not in favour of chemotherapy and an inexperienced developer, together with a marginal therapeutic effect, limited the commercial potential of amantadine. The lack of effect against influenza B was also a negative factor despite the preponderance of influenza A. Furthermore, not all type A viruses were found to be effectively treated (Lubeck, *et al.*, 1978).

More recent development of inhibitors of the influenza virus neuraminidase has produced two potent drugs, zanamivir and oseltamivir, both active against both A and B influenza. So far there is less evidence of resistance to these two new agents than for amantidine (Gubareva, 2004). Despite this, sales of oseltamivir in 2002 were only approximately £200 million (which in itself was a 184% increase from the previous year) and factors such as increased vaccination do not explain this poor use of what are good drugs. These sales figures were obtained during an interpandemic period – one may only speculate what the sales could be during a pandemic. However, unless a pandemic could be anticipated or planned for (by stockpiling drugs) it is unlikely that compound supply could keep pace with patient demand.

Influenza is a disease with a relatively short duration of viral replication, most of which is complete when therapy is initiated. At the time of therapy, the clinical signs of the disease are thought to be largely mediated by the effect of induced cytokines. This means that, to be effective, the neuraminidase inhibitors need to be used within 48 h of the appearance of clinical signs. This has proven difficult for most patient populations and their physicians. Additionally, there is a general false perception that influenza virus infection is a trivial disease similar to the common cold. Furthermore, at the early stages, symptoms

Table 3. Antiviral drugs against respiratory viruses

Infectious agent	Generic name	Trade name	Type of inhibitor
Influenza A	Amantadine	Virofral®	M2 protein binding
Influenza A	Rimantadine	Flumadine®	M2 protein binding
Influenza A and B	Zanamivir	Relenza®	Neuraminidase inhibitor
Influenza A and B	Oseltamivir	Tamiflu®	Neuraminidase inhibitor
RSV	Ribavirin	Virazole®	Nucleoside acting on cellular function?
RSV	Palivizumab	Synagis®	Monoclonal antibody

RSV, respiratory syncytical virus.

are often difficult to differentiate from those of other respiratory diseases such as the common cold or adenovirus infections, thus suggesting the need for a rapid point of care diagnostic, which increases the complexity and cost of treatment. There certainly is room for chemoprophylaxis, especially in closed communities such as homes for the elderly, but therapy will result in only a minor reduction in the overall disease burden. In case of a new more serious influenza epidemic, therapy and prophylaxis will have important roles until vaccine production is sufficient.

A combination treatment directed against both influenza virus replication and symptoms might be developed but, to our knowledge, such a combination has not been reported. It might even be possible to inhibit both replication and symptoms by blocking cellular pathways such as NF-κB signalling (Nimmerjahn *et al.*, 2004).

Rhinovirus

Rhino virus infection has created a lot of interest in the marketing departments of the pharmaceutical industry due to the very large number of potential customers. There is no drug on the market licensed for treatment of rhinovirus infections despite several good targets for selective antiviral drugs.

Once again, a major complication is the short duration of rhinovirus replication, largely completed when the patients have developed recognizable signs. The diagnostic problem is substantial since a number of infectious agents can cause the symptoms of common cold and, as with influenza, the time from symptoms and diagnosis to initiation of treatment has to be very short.

A number of inhibitors, for example WIN54954, are often referred to as a canyon-binding inhibitors due to their mechanism of action, which entails interaction with rhinovirus structural protein VP1 either preventing attachment or uncoating. The precise mechanism of inhibition of rhinovirus by the canyon-binders depends on whether the virus VP1 binds to the major receptor for rhinovirus ICAM-1, or, in a small number of virus strains, to the minor receptor low-density lipoprotein receptor (Shepard *et al.*, 1993; Zhao *et al.*, 1996). WIN 54954 was evaluated in clinical trials and, despite potent anti-rhinovirus activity, there was no detectable therapeutic benefit which, at the time, was thought to be due to poor delivery of the compound to the site of replication (Turner *et al.*, 1993). It was also discovered that viral resistance was likely to be of significance. Indeed, in the laboratory it was even possible to select viruses whose replication was not only resistant to the WIN compounds but actually required them to replicate efficiently (Wang *et al.*, 1998).

Other more recent compounds in the canyon-binding class or those targeting the rhinovirus protease appear to have stalled whilst still in development. Indeed, recent trials with pleconaril (a canyon-binding inhibitor) indicate that treatment of rhinovirus infections not only resulted in only a 1 day reduction in clinical signs but also induced levels of cytochrome P-450 3A enzymes (Hayden *et al.*, 2003). It is also possible that another reason for the lack of progression of rhinovirus drugs is the perception of a poor commercial opportunity, enhanced by the recent experience with influenza virus inhibitors.

As for other viral infections characterized by a short duration of viral replication and symptoms lasting only a few days after viral replication, combination therapy might be a possibility. One clinical study combining IFN with two anti-inflammatory agents has shown significant efficacy in patients with rhinovirus infection (Gwaltney *et al.*, 2002).

Respiratory syncytical virus

Respiratory syncytical virus (RSV) causes bronchiolitis and is a common cause for hospitalization of infants and has been reviewed by Torence & Powell (2002). The only therapy presently available for all patients is ribavirin given as an aerosol. The antiviral effect of this treatment is debatable and is not without side effects.

A rapid diagnosis would be necessary to be able to initiate an antiviral treatment early enough to give clinical benefit. More recently, a humanized therapeutic monoclonal antibody, palivizumab, has been developed which is approved in the USA for prophylaxis of at-risk neonatal populations and is very effective in this population (Fenton *et al.*, 2004). The development of novel small molecule inhibitors of RSV is limited by the lack of attractive molecular targets in the RSV genome (with the exception of the RSV polymerase). Nevertheless, there has recently been some progression with the identification of inhibitors of viral uncoating which have completed Phase I clinical trials (Arrow Therapeutics Ltd, London, UK: press release, 28 June 2004). It remains to be seen if these exciting compounds will progress to the marketplace but the medical need of safe, small-molecule drugs for the treatment of RSV is compelling.

Adenovirus

A significant proportion of 'common cold infections' are caused by adenovirus. In addition, in the immunocompromised population, infection by adenovirus can be life-threatening (Carrigan, 1997). As an interesting aside, the significance of adenovirus infection is illustrated by the attention it is given by the US military, who have seen several outbreaks caused by the concentration of large numbers of 'raw recruits'. This led to the US army using a vaccine against adenovirus in its boot camps. However, the major source of these vaccines is no longer in production and hence adenovirus infection is again becoming a

problem (Kolavic-Gray *et al.*, 2002). These facts would imply a clinical and societal need for anti-adenovirus agents. Indeed, the adenovirus-encoded DNA polymerase would appear to be an attractive target and several compounds have been reported to be inhibitors of this polymerase and of adenovirus replication (Mental *et al.*, 2000). However, the fact that the clinical need for an adenovirus inhibitor is diffuse and unclear has not attracted pharmaceutical companies to work in this area. We might also anticipate the same issues as shown for inhibitors of influenza and rhinoviruses, that is, the need for diagnosis and early treatment.

HIV/AIDS

The drugs currently licensed for use against HIV are shown in Table 4. The HIV pandemic has been the major stimulus of the rapid development of new antiviral drugs over the last decade. Although discovery of inhibitors of HIV started by screening in cell culture with the available nucleoside analogues from previous antiviral programs, development of new drugs today is largely rational and based on structure information on viral enzymes and proteins.

Development of the first anti-HIV drug, zidovudine (AZT) was extremely rapid, facilitated by the fact that the compound was already available and identified as an inhibitor of retrovirus replication. Due to the serious nature of the infection, there was no need for efficacy studies in animals and the requirement of only limited Phase II results (Fischl *et al.*, 1987) was sufficient for FDA approval. The reverse transcriptase (RT) of HIV was found to be a suitable target for antiviral drugs and several nucleoside and nucleotide analogues acting as competitive substrates terminating DNA synthesis have been developed, as shown in Table 4. Allosteric RT inhibitors [non-nucleoside reverse transcriptase inhibitors (NNRTIs)] were found to be more potent *in vitro* than the nucleoside and nucleotide analogues and also better suited to a rational design based on structure–activity relationship (SAR). The improved SAR of the NNRTIs is due to the direct inhibition of RT activity, in contrast to nucleoside and nucleotide analogues, which require phosphorylation by cellular kinases in order to inhibit. However, it was soon found that inhibition of HIV NNRTIs resulted in the rapid emergence of resistance *in vitro* and *in vivo*. This weakness caused a loss of interest in the NNRTIs until it was shown that when used in combination with a NRTI, not only did the rapid emergence of resistance diminish but the compounds could act synergistically (Larder, 1992).

Inhibitors of the HIV protease emerged based upon rational design starting from the natural protease substrates. These inhibitors were shown to be highly potent and to rapidly increase the survival of HIV/AIDS patients.

Table 4. Antiviral drugs against HIV

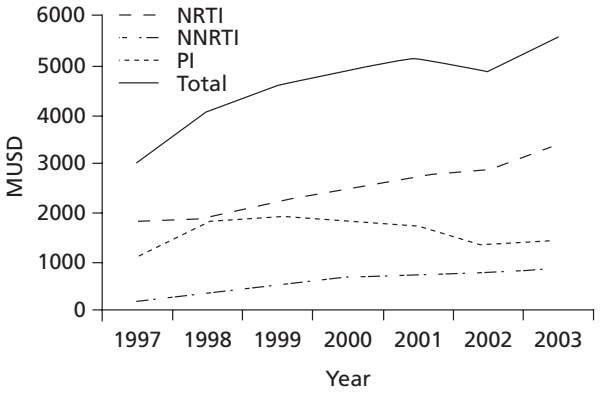
Generic name	Trade name	Type of inhibitor
Zidovudine	Retrovir®	NRTI
Didanosine	Videx®	NRTI
Zalcitibine	HIVID®	NRTI
Lamivudine	Epivir®	NRTI
Stavudine	Zerit®	NRTI
Abacavir	Ziagen®	NRTI
Tenofavir	Viread®	NRTI
Emtricitabine	Emtrive®	NRTI
AZT/3TC	Combivir®	2 NRTIs
AZT/3TC/abacavir	Trizivir®	3 NRTIs
Emtricitabine/tenofovir	Truvada®	2 NRTIs
Abacavir/lamivudine	Epzicom®	2 NRTIs
Nevirapine	Viramune®	NNRTI
Delaviridine	Rescriptor®	NNRTI
Efavirenz	Sustiva®	NNRTI
Saquinavir	Invirase®, Fortovase®	PI
Ritonavir	Norvir®	PI
Indinavir	Crixivan®	PI
Nelfinavir	Viracept®	PI
Amprenavir	Agenerase®	PI
Lopinavir/ritonavir	Kaletra®	2 PIs
Atazanavir	Reyataz®	PI
Fosamprenavir	Lexiva®	PI prodrug
Efuviritide	Fuzeon®	Fusion inhibitor

AZT, zidovudine; 3TC, lamivudine; NRTI, nucleoside reverse transcriptase inhibitor. NNRTI, non-nucleoside reverse transcriptase inhibitor. PI, protease inhibitor.

However, resistance development and side effects have limited the usefulness of all classes of HIV inhibitors including NRTIs, NNRTIs and protease inhibitors (Wainberg, 2004; Rhee *et al.*, 2004). The newly emerging inhibitors of other viral functions such as fusion, viral co-receptor and integration are also likely to have limitations with respect to resistance development (Greenberg & Cammack, 2004) and toxicity.

The increasing number of anti-HIV drugs with different resistance patterns and toxicity profiles allows several effective combinations to be used. The sequence in which such combinations should be used is important in order to maximize the benefit to the patients. The optimal use of combinations has been outlined by the Infectious Disease Society of Medicine (Aberg *et al.*, 2004), by the British HIV Association (Pozniak *et al.*, 2003; http://www.bhiva.org), in the NIH guidelines of 29 October 2004 (http://AIDSinfo.nih.gov) and in the European Guidelines (Murphy & Gazzard, 2003). Despite the impressive development of anti-HIV drugs, there will be a constant need for drugs with new patterns of resistance, a slower rate of resistance development, easy admin-

Figure 2. HIV drug sales 1997–2003



MUSD, millions of US dollars; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.
Source: DATAMONITOR - Market Dynamics: HIV; DMHC1854 (January 2003). DATAMONITOR - Commercial Insight: HIV; DMHC2007 (August 2004).

istration and fewer side effects. This need will be fulfilled as long as the pharmaceutical industry finds the HIV field commercially attractive. The attractiveness of HIV for pharmaceutical companies is illustrated in Figure 2, which shows the sales of HIV drugs between 1997 and 2003.

The lack of an effective vaccine against HIV infection or disease is one of the most serious medical problems in view of the still rapidly expanding HIV epidemic. Vaginal microbicides are being evaluated as a means of preventing HIV transmission and, most importantly, as a protective measure controlled by women. Although it seems likely that such an approach will have clinical benefit, an effective licensed formulation is still several years away. NNRTIs seem to be especially suited for microbicides since some can directly inactivate HIV RT in virus particles as well as inhibit replication in cells (D'Cruz *et al.*, 2004).

The HIV market is growing both in the number of new patients being infected and due to the increased survival in a chronic disease. The market is also changing due to development and spread of resistant virus. In view of the lack of prospect of effective vaccines, this situation seems likely to last. Changed behaviour and appearance of effective microbicides will hopefully have an impact on the epidemic by reducing transmission, but the need for new treatments will remain.

Hepatitis viruses

Drugs licensed for the treatment of hepatitis viruses are shown in Table 5.

HBV

Approximately 250 million people are infected with HBV. Many of these are asymptomatic carriers who show no apparent symptoms during their lives but who may infect their children *in utero*. When an individual is undergoing an active HBV infection, the disease follows a progressive course starting with inflammation of the liver followed fibrosis to sclerosis and finally hepatocellular carcinoma. Death from hepatocellular carcinoma is one of the most common forms of human cancer-related deaths.

HBV disease was one of the first viral diseases for which IFN therapy was shown to be effective. Therapy is continued for up to 2 years and, overall, approximately 25% of patients obtain a long-term benefit. However, this

Table 5. Antiviral drugs against hepatitis viruses

Virus indicated	Generic name	Trade name	Inhibitor type
HBV	Interferon	IntronA® and others	Immunomodulator
	Lamivudine	Zeffix®, Heptovir®	Nucleoside analogue polymerase inhibitor
	Adefovir	Hepsera®	Nucleotide analogue polymerase inhibitor
HCV	Interferon and pegylated interferon	IntronA®, Roferon®, Infergen®, PEG-Intron®, Pegasys®	Immunomodulator
	Interferon or pegylated interferon plus ribavirin	Rebetron®, PEG-Intron/ Rebetol®, Pegasys/Copegus®	Immunomodulator plus nucleoside analogue
	Ribavirin		Nucleoside analogue

HBV, hepatitis B virus; HCV, hepatitis C virus.

benefit is not without significant cost. Interferon has to be injected and has severe side effects including all of the symptoms of a classic influenza virus infection (fever, aching muscles and nausea) and has even been shown to be associated with increased levels of suicide (Brenard, 1997). Finally, the treatment is relatively expensive and, for the reasons described above, not very successful. Recently, pegylated IFN has been introduced into the clinic which reduces the frequency of injections whilst at the same time increasing its overall efficiency (Craxi & Cooksley, 2003).

The large number of potential patients and the severity of disease plus the unmet medical need led several companies to search for small-molecule inhibitors of HBV replication. Currently, only one well-characterized molecular target is known. namely the HBV polymerase. This enzyme has RT as well as DNA polymerase activity and in many ways resembles the HIV RT. In the 1990s, a nucleoside analogue FIAU was being developed for treatment of HBV disease. However, during clinical trials it was shown to have caused the death of a number of patients thereby halting its further development (Brahams, 1994). The mechanism for the toxicity is thought to be due to the incorporation of the monophosphate into mitochondrial DNA and with subsequent effects on lactic acid levels and metabolism. It was a further 5 years before the next nucleoside analogue was developed for HBV. The compound lamivudine was in the later stages of development for HIV and had been shown to be a potent inhibitor of HBV replication in cell lines. Subsequent clinical trials showed that it had a potent effect on HBV plasma levels and hence replication. Approximately 15% of patients showed significant improvement within 1 year including seroconversion to e antigen positivity, indicative of suppression of virus replication. A further 15% benefited after a subsequent year on therapy (Lai *et al.*, 1997). However, a significant number of patients develop genotypic, and sometimes, phenotypic resistant virus during therapy which in many cases leads to the withdrawal of therapy. Thus, whilst lamivudine has proven to be a safe and effective treatment for HBV infection, it is used in a limited number of HBV patients. Limitations include the contraindication in treating patients with advanced liver disease who may undergo an immune flare. Most importantly are the economic realities in the countries with high HBV infection rates, such as Africa and SE Asia, which have a popular use of traditional medicines.

Despite these challenges, there is significant research into improved HBV compounds. The compound adefovir has been launched for the treatment of HBV in several territories and, in combination with lamivudine, has an improved resistance profile, albeit with a corresponding increase in toxicity (James, 1999). Other compounds include emtricitabine, entecavir and telbivudine. In all of these cases, it will be intriguing to observe the practical commercial opportunities when they are launched. The HBV field has recently been reviewed by Zoulim (2004).

HCV

In the 1990s, it became apparent that, after accounting for hepatitis A and B infections, there remained a large number of so-called nonA–nonB hepatitis virus infections. Major efforts were made both in academia and industry to identify the causative agent and eventually a breakthrough was made in cloning HCV from serum from a human donor (Choo *et al.*, 1989). It is now known that some 170–200 million people are infected with HCV (around 3% of the world's population). Indeed, in some countries, the infection rate is much higher and it is also the major single cause of the need for liver transplants.

HCV is a member of the Hepacivirus genus (which also contains agents such as Dengue virus and yellow fever virus) and has an RNA genome with several virus-coded enzymes, which by previous experience should be attractive targets, such as the viral polymerase (NS5b), protease (NS3/4a) and helicase (NS3). In addition, several less well precedented targets may be considered such as the virus internal ribosome entry site (IRES) and the NS2 zinc-dependant protease.

Initially, IFN was used for treatment of HCV with some success. However the classic side effects coupled with the lack of activity on HCV type 1 (the major type found in HCV patients in the US and Europe) has limited its usefulness. A pegylated formulation of IFN has been developed to decrease the frequency of injections and hence reduce the side effects. In addition, it was found that by co-administrating oral ribavirin, the effect of IFN could be enhanced. The mechanism by which ribavirin enhances the activity of IFN is not entirely clear, however recent findings would suggest that the major effect is due to the compound inhibiting the host enzyme inosine monophosphate dehydrogenase thus perturbing intracellular GTP pools (Leyssen *et al.*, 2005). However, even though the combination of pegylated IFN and ribavirin has improved therapy of HCV, there is still a huge medical need and commercial opportunity for small-molecule-specific inhibitors of HCV replication (Foster, 2004).

Initial efforts in drug discovery were frustrated by the lack of a replicative system for HCV and the lack of tractability of the targets. High throughput screening using the three principle targets has been largely unsuccessful. The helicase has proven particularly challenging with no molecule in development as yet. The HCV protease was initially considered to be an almost impossible target for the development of inhibitors. The structure of the enzyme has been described as largely featureless and not amenable for inhibition by a small molecule (DeFrancesco *et al.*, 2003; Dymock *et al.*, 2000).

Recently, inhibitors of HCV protease have been developed which are both potent and selective (Lamarre *et al.*, 2003). Proof-of-concept trials have shown BILN 2061 to have remarkable activity, reducing virus load by 2–3 $\log_{10}$ within 2 days of therapy. Although this compound is currently on hold due to the appearance of cardiac toxicity in 4-week studies in animals (Hinrichsen *et al.*, 2004), it has proven a catalyst for many companies to re-establish efforts on HCV protease which should eventually give rise to a generation of clinically useful agents. Additional support for the attractiveness of HCV protease as a target for therapy of infection is given by the observation that it also has the ability to reduce the response to IFN due to blockage of IRF-3. Thus, an inhibitor of HCV protease might, in addition to blockading viral replication, also enhance interferon response (Foy *et al.*, 2003).

The HCV polymerase has also been studied as a target. Initially, progression was slow because the lack of a cell-based system for the replication of HCV meant that it was difficult to test nucleoside analogues for activity. The construction of a cell-based HCV replicon by Lohmann *et al.* (1999) has facilitated the study of nucleoside analogues against HCV replication. Several nucleoside analogues are now in early development as inhibitors of HCV. In addition, high throughput screening has identified several NNRTI inhibitors of HCV polymerase. These inhibitors are potent but several of them appear to inhibit limited subsets of the six HCV types, which would greatly limit their usefulness (Sarisky, 2004). Inhibitors of HCV polymerase have recently been reviewed (Beaulieu & Tsantrizos, 2004). Resistance development is expected to be a clinical problem requiring combination therapy, in view of the large pool of rapidly replicating and error-prone pool of HCV carried by each patient (Simmonds, 2004; Lu *et al.*, 2004).

Other potential inhibitors of HCV replication in development include compounds active against the IRES element and antisense inhibition. Little progress has been reported for the inhibition of the helicase or zinc dependent protease.

Human papilloma virus (HPV)

HPV is the cause of skin warts in humans including on the genitals of both men and women. Some high-risk types of HPV, for example, types 16 and 18, are strongly associated with conditions, such as vaginal erosions and dysplasia eventually leading to cancer of the cervix. HPV prevalence in the female's genital tract can be as high as 46% in the most at-risk groups. Over a million new cases of condyloma acuminata are reported annually, making HPV the most common sexually transmitted disease in the USA and UK (Gall, 2001).

There are no selective antiviral therapies for HPV but a number of approaches are in use (Bernard, 2004). The current treatment of genital warts appears somewhat barbaric, consisting of physical agents such as surgery, liquid nitrogen and toxic agents such as podophylotoxin. 5-Fluoruracil has also been used (without FDA approval) as a 5% topical cream; however, it is associated with significant side effects. Despite their radical nature, the treatments are largely ineffective with around 50% of patents requiring repeated therapy. Recently, Imiquimod, a topical formulation which acts by locally inducing IFN and other cytokines, has been developed and has reduced recurrence rates between 13–19% (Edwards *et al.*, 1998). However, there is still a large clinical need for selective therapies of HPV infection (Gall, 2001; Bernard, 2004).

HPV is a small virus with only one molecular target (a DNA helicase) that could be considered attractive for developing inhibitors. This enzyme has proven technically difficult to express and to establish in assays and as yet no inhibitors have been developed. Recent approaches taken are more directed towards the immune response with a variety of therapeutic vaccines in development largely, but not exclusively, targeted towards the high-risk oncogenic types of HPV. Topical cidofovir has also been used in clinical trials in HIV patients (Snoeck *et al.*, 2001). Currently, there is an urgent need for effective vaccines and non-toxic/virus-specific treatments.

Bioterrorism

In recent years, the potential use of viruses (and other infectious agents) as weapons in bioterrorism has received attention within governments and the media. In particular, the possible use of smallpox has attracted much debate and stimulated governments to procure stockpiles of suitable vaccines and, in some cases, to undertake limited vaccination of at-risk groups such as health workers. However, there are considerable issues with the use of vaccinia in mass vaccination campaigns due to potential side effects and the fact that many patients, such as those who are immunocompromised or have eczema, are contraindicated for vaccination. In many ways, a small-molecule inhibitor with a long shelf life is a more attractive proposition than vaccination as it allows rapid use targeted towards foci of infection. Considerable research has been undertaken into the identification of new anti-smallpox compounds. In laboratory experiments, adefovir has been shown to have potential use, however it is not known if it is a truly specific inhibitor of smallpox and, in studies in both HIV and HBV, it has been shown to have adverse events (James, 1999). In addition, the inability to test any new inhibitor of smallpox in clinical trials will make their use an act of faith. It is interesting to recall that the first clinical trials of an

antiviral compound were undertaken in the 1960s with the compound 1-methylisatin-3-thiosemicarbazone (Marboran), an inhibitor of smallpox. In a clinical trial in Madras, Marboran was shown to be effective when used prophylactically for the prevention of smallpox infection and reduction of consequential death (Bauer, 1965). The compound was found to be associated with side effects including reversible liver toxicity but Marboran analogues could be a very credible starting point for a new programme.

Apart from smallpox (with its heavy emotive reputation) there have been very few other viruses consistently identified as potential bioweapons. Potentially, these agents could include viruses such as Ebola or other haemorrhagic viruses. However, the production of viruses is technically demanding and the production of a form suitable for use as a weapon even more so. Hence it is difficult to assess the nature of the real threat of viruses in the future. Potentially, inhibitor programmes on these exotic viruses could be based upon other more orthodox ones such as HCV or RSV. This will require close cooperation between industry and the defense organizations to arrange the screening and development of such inhibitors.

Emerging viral infections

In this section we will limit our discussion to a brief description of potential areas for development outside the mainstream diseases. The history of antiviral research has examples of emerging viruses such as HIV or HCV. Such emerging viruses may pose considerable problems to healthcare. The changes in human behaviour in the last part of the 20th century cannot be understated and has led to a dramatic increase in the potential of emerging viral infections. Expansion of the traditional areas of habitation has exposed the population to agents such as Sin Nombre virus. Similarly, mass long-distance travel opens up the possibility of rapid widespread dissemination of what would be otherwise local infections (arguably the spread of HIV in the 1980s was such an event). In the future, societal activities such as sex tourism will have a major effect on the transmission of new infections. Above all, the emergence of so-called 'super cities' such as Shanghai and Mexico City could act as reservoirs, incubating and expanding new agents which could emerge in a explosive manner on the unprotected international population. As described above, this may be of particular importance in the emergence of new strains of influenza virus such as H5N1.

It is difficult to predict where and when such agents may emerge in the future and the consequences. For example, the recent severe acute respiratory syndrome (SARS) virus was not anticipated and whilst it caused considerable impact on healthcare in countries as widespread as China and Canada and the infection did appear to have a high mortality rate, the virus was controlled by traditional healthcare practices such as barrier nursing and isolation. The SARS agent has not re-emerged since 2003 with the exception of an infected laboratory worker in China. It remains to be seen if therapies or vaccines will be needed for SARS, but it is clear that when a new virus emerges there can be severe economical consequences, even when rather few patients are involved.

Of much more concern is the possible emergence of a new pandemic strain of influenza virus (H5N1 or similar) with enormous implications and potentially leading to many millions of deaths. The production of suitable vaccines would take several months, allowing the strain to spread and cause major morbidity and so it would seem prudent to consider the stockpiling of significant quantities of the new neuraminidase inhibitors (Oxford *et al.*, 2004) which can inhibit all strains of influenza and could be used to limit the spread and consequences of infection whilst a vaccine is produced.

Infections such as West Nile fever could emerge as a consequence of changes in climate. Human contact with animals carrying viral infections previously not transmitted to humans is a real risk, exemplified by HIV. Research in academic organizations on viruses presently not attractive as targets for drug-development programmes in industry gives a basis for future drug development, should a market develop. However, as in bioweapons research, there is a need for co-operation between industry and academia in development of such compounds. Indeed, if development of compounds against agents such as West Nile fever is to occur, it will require significant governmental support, perhaps in the form of dedicated institutions containing the expertise found in industry necessary for drug discovery and development.

Conclusions

The development of antiviral drugs over the next 20 years is likely to be focused on HIV and HCV in view of their medical importance and a continuous need for new drugs to cope with resistance development.

Antiviral research is also more likely to be centred on novel therapies such as therapeutic vaccines rather than on new diseases. One avenue which has great potential is the use of disease-modifying treatments acting on host targets in combination with inhibitors of virus replication. For example, the use of an inhibitor of HCV along with a treatment for fibrosis could have major impact on liver disease. One such approach using an inhibitor of herpes simplex (aciclovir) in combination with an anti-inflammatory agent has already been shown to lead to significant improvements in the treatment of herpes labialis infections. Viruses (including HPV, HBV, HCV and EBV) establishing latent

or chronic infections by blocking apoptosis through stimulation of P13k-Akt-mediated cell survival might be treated by interfering with cellular functions (Cooray, 2004). Such approaches may have enormous potential and could revolutionize our approaches to the treatment of virus infections, but will require relevant *in vivo* models to predict clinical effect. The antiviral field began when virus-coded targets were found and the next phase might be when the use of these targets are combined with cellular targets.

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