Antiviral drugs – a short history of their discovery and development

Hugh J. Field and Erik De Clercq

For many years it was believed that there were no effective antiviral drugs. Since the late 1950s scientists have made great progress in this area as Hugh Field and Erik De Clercq describe.

The concept of specific antiviral therapy and a false dawn

The 1946 edition of van Rooyen and Rhodes' *Virus Diseases of Man* introduced the concept of specific therapy for a number of virus infections, including mumps and smallpox. This early work focused on the use of the current bacterial antibiotics, including sulphonamides. The futility of these early attempts led to the dogma that viruses are not susceptible to 'antibiotics' and for two decades virologists were taught that selective toxicity for these obligate intracellular parasites was unattainable. Several lines of research were to overturn this *idée fixe*. In 1957 came the famous first description by Isaacs and Lindenmann of interferon. Human interferons were subsequently developed for the treatment of particular virus infections, i.e. hepatitis B and, more recently, hepatitis C virus infections, as pegylated interferon, combined with ribavirin. However, the discovery of the interferons in the late 1950s was something of a false dawn.

Idoxuridine: the first useful antiviral nucleoside analogue

Many in the antiviral field recognize as a most important early milestone the description of 5-iodo-2′-deoxyuridine (idoxuridine, IDU) by Dr Bill Prusoff in 1959 and the realization of its antiviral properties. The first publications on this and similar nucleoside analogues appeared in cancer journals and it is clear that the aim was to develop molecules to interfere with DNA synthesis in order to produce cytostatic or cytotoxic drugs for the treatment of neoplastic disease. However, an important by-product of this work was the discovery that IDU was a specific inhibitor of certain large DNA viruses, most notably herpes simplex virus (HSV). The compound is cytotoxic and was therefore only suitable for topical application, for which it remains in use to the present day. The development of IDU from laboratory inhibitor to useful antiviral drug was driven by several notable pioneers, especially the ophthalmologist Dr Herbert Kaufman who proved its clinical value in 1962 and, subsequently, that of trifluorothymidine (TFT) in 1964.

The first description of the antiviral activity of adenine arabinoside (vidarabine, ara-A) by M. Privat de Garilhe and J. De Rudder also dates from the avant-garde year 1964. Ara-A was the first of the nucleoside analogues to be sufficiently non-toxic to be given systemically and the work of Dr Richard Whitley proved beyond doubt the clinical value of this compound, showing for the first time that, providing treatment was commenced early in the disease, it was possible to curtail herpes zoster in the immunosuppressed and reverse the potentially lethal progression of herpes encephalitis and the overwhelming herpes infections that occasionally occur in the newborn.

Selected milestones in antiviral drug development

1951	β-Thiosemicarbazone	Hamre *et al.*
1957	Interferon	Isaacs & Lindenmann
1959	IDU	Prusoff
1961	Hydroxybenzylbenzimidazole	Tamm & Eggers
1961	Guanidine	Barrera-Oro & Melnick
1962	IDU (clinical effectiveness)	Kaufman
1963	Marboran (clinical effectiveness)	Bauer *et al.*
1964	TFT (clinical effectiveness)	Kaufman
1964	Amantadine	Davies, Hoffmann *et al.*
1964	Ara-A	Privat de Garilhe & De Rudder
1972	Ribavirin	Sidwell, Robins *et al.*
1976	Ara-A (clinical effectiveness)	Whitley
1977	Acyclovir	Elion, Schaeffer, Collins & Bauer
1978	DHPA	De Clercq & Holy
1979	Phosphonoformic acid (PFA)	Helgstrand & Öberg
1979	BVDU	De Clercq *et al.*
1982	Ganciclovir	Verheyden & J.C. Martin
1985	Azidothymidine (AZT)	Mitsuya, Broder *et al.*
1986	ddI, ddC, . . .	Mitsuya & Broder
1986	Adefovir (PMEA)	De Clercq, Holy *et al.*
1987	Cidofovir (HPMPC)	De Clercq, Holy *et al.*
1989	Famciclovir (oral prodrug strategy)	Harnden, Vere Hodge *et al.*
1989 1990	HEPT/TIBO	De Clercq, Baba, Pauwels & Janssen
1990	Saquinavir	J.A. Martin, Roberts *et al.*
1991	3TC	Belleau *et al.*
1993	Tenofovir (PMPA)	Balzarini, De Clercq & Holy
1993	Relenza	von Itzstein *et al.*
1997	Oseltamivir	Kim *et al.*

Poliomyelitis, smallpox: important early antiviral targets

We will return to herpes antivirals, but first we should remember the origins of several other lines of antiviral research and the early pioneers. Poliomyelitis was still a serious threat in the developed world when guanidine and 2-(α-hydroxybenzyl)benzimidazole (HBB) were shown to be specific inhibitors of this small, positive-strand picornavirus and other picornaviruses (i.e. Coxsackie and Echo) as early as 1961 by J.G. Barrera-Oro and Joe Melnick, and Igor Tamm and Hans Eggers, respectively. The latter promoted the concept of specific antiviral therapy for polio and their lectures and writing much influenced the field in the 1960s, although, in the event, polio was eventually controlled by vaccination rather than chemotherapy. Another important virus threat was that of smallpox caused by variola virus, which is among the largest of all viruses with a double-stranded DNA genome comprising more than 200 genes. The compound β-thiosemicarbazone was first reported to be an inhibitor of a related poxvirus, vaccinia virus, in 1951 by D. Hamre, K.A. Brownlee and R. Donovick. Dr John Bauer at the then Wellcome Foundation Laboratories in Beckenham, UK, led the team which developed the drug marboran, a β-thiosemicarbazone derivative. Marboran was shown in several trials to have some clinical efficacy both for the treatment of smallpox and the complications of vaccinia following vaccination. Furthermore, it was shown that marboran was a highly effective agent for chemoprophylaxis in the management of smallpox contacts. Smallpox was shortly to be eradicated by means of the WHO vaccination scheme and work on marboran ceased. However, recently there has been renewed interest in antipoxvirus agents as a result of the threat of smallpox being reintroduced by an act of terrorism.

ABOVE:
Picture taken at the 10th International Conference on Antiviral Research, Atlanta, 6–11 April 1997. Many of those in this photograph have made a major contribution to the development of antivirals.
1, G.L. Galasso; 2, Mrs Galasso; 3, W.H. Prusoff; 4, E.R. Kern; 5, R.F. Schinazi; 6, J.C. Martin; 7, G.B. Elion; 8, R.J. Whitley; 9, H.J. Field; 10, J.-L. Imbach; 11, Mrs Shigeta; 12, S. Shigeta; 13, D.C. Liotta; 14, J.A. Secrist III; 15, J.-C. Graciet; 16, L.J. Stuyver; 17, D. Parker; 18, C.R. Cusick; 19, K. Shockley; 20, B. Öberg; 21, K.Y. Hostetler; 22, A. Kwong; 23, C. McGuigan; 24, R.W. Sidwell; 25, K.K. Biron; 26, J.W. Mellors; 27, E. De Clercq; 28, P.D. Griffiths; 29, L.M. Mofenson; 30, J.-P. Sommadossi; 31, A. Molla; 32, D. Schaeffer; 33, C. Laughlin; 34, N. Bischofberger.
COURTESY DR RAYMOND F. SCHINAZI, EMORY UNIVERSITY, GA, USA

Treatments for influenza virus infections

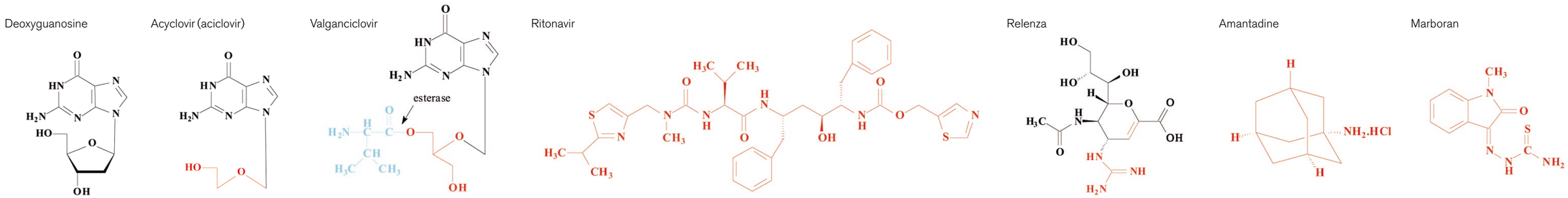
Influenza was also an early antiviral target, and in 1964 it was reported by C.E. Hoffmann and co-workers that amantadine was a specific inhibitor of the negative RNA strand virus, influenza A. Amantadine and its sister compound, rimantadine, were later shown to act by interaction with the viral M2 protein which forms an ion channel during the early stages of virus replication. As for almost all other specific antiviral agents, resistant mutants were obtained by passage of virus at sub-inhibitory concentrations of the inhibitor and in this case these mutations mapped to the M2 and haemagglutinin (HA) genes. Furthermore, when the compounds were used clinically, resistance developed quickly in patients and this was one of the factors which argued against their widespread clinical use. Rimantadine was, however, widely used clinically in Eastern Europe during the cold-war years, although the data relating to its use in man were not so easily forthcoming. However, the work on amantadine and rimantadine provided a platform for the development of the next generation of anti-influenza drugs that resulted from a programme of rational drug design. The crystal structures of the influenza envelope proteins – HA and neuraminidase (NA) – were solved. Influenza NA was known to interact with sialic acid residues on host-cell plasma membranes and the first of several sialic acid analogues, Neu5Ac2en, designed by M. von Itzstein and his colleagues in 1993, based on the crystal structure of influenza NA, led to the development of 4-guanidino-Neu5Ac2en (relenza), the first NA inhibitor, to be marketed by Glaxo. A difficulty with this compound is its poor oral bioavailability, necessitating its application by means of inhalation. Other NA inhibitors tailored on the sialic acid residue followed, including the successful oseltamivir (developed at Gilead sciences and first reported by C.U. Kim *et al.* in 1997) in which a cyclohexene ring was introduced and a polar glycerol replaced with a more lipophilic side-chain. Furthermore, oseltamivir is an ethyl ester that is orally bioavailable and readily converted to the active carboxylate by esterases in the liver, a prodrug approach first used for the antiherpetic compound famciclovir (see below).

The first broad-spectrum antiviral after interferon

Ribavirin was reported in 1972 by Robert Sidwell, R.K. Robins and their colleagues as a broad-spectrum antiviral acting against many different virus families, notably the negative RNA strand virus, respiratory syncytial virus. It was noted that virus resistance to ribavirin was not detected for any of the susceptible virus families. This may be related to the fact that ribavirin primarily targets a host-cell protein, and, indeed ribavirin (5′-monophosphate) has been found to inhibit IMP dehydrogenase, the enzyme responsible for the conversion of IMP to XMP. Another discovery in the 1970s was the antiherpesvirus activity of the pyrophosphate analogue phosphonoformic acid (phosphonoformate PFA) following the earlier discovery of the lead compound, phosphonoacetic acid (PAA). PFA, described by B. Öberg and developed at the Swedish pharmaceutical company, Astra, suffers from toxicity problems, including nephrotoxicity. However, PFA continues to have a role in managing HSV infections in immunocompromised patients who are resistant to the classical antiherpetic compounds (e.g. acyclovir).

The advent of acyclovir

No history of the origins of antivirals would be complete without acknowledging the enormous impact of the compound acyclovir. Like IDU, acyclovir was the result of a drug development programme not primarily aimed at antivirals. The names of Dr Gertrude (Trudy) Elion



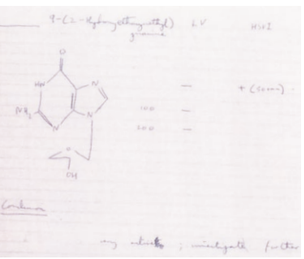
ABOVE:
The chemical structure of a selection of antiviral compounds. Red is used to indicate those parts of antiviral compounds which differ from the natural structure from which they are derived. Blue is used to depict the components of prodrugs that are removed by enzymic action *in vivo* to yield active compounds.
A more complete set of structures accompanies the online version of this article at www.sgm.ac.uk

and Dr Howard Schaeffer (Burroughs Wellcome, USA) are inextricably linked to this compound, although its potent antiviral properties were first uncovered by Drs Peter Collins and John Bauer at the Wellcome Laboratories (UK) where the compound had been sent for antiviral activity evaluation. Dr Bauer coined the term acycloguanosine, although this was subsequently dropped in favour of the generic term acyclovir (aciclovir). Dr Elion and her colleagues produced a definitive mechanism of action and the thoroughness of this work and the associated pharmacological data were crucial to the early acceptance of the compound. Acyclovir was shown to be a substrate for the HSV-encoded deoxyribopyrimidine kinase, usually called thymidine kinase (TK). Acyclovir monophosphate is then further phosphorylated by cellular kinases and the resulting acyclovir triphosphate is a potent suicide inhibitor of the herpes-specified DNA polymerase. The fact that a (deoxy)-guanosine analogue serves as a substrate for the virus deoxyribopyrimidine kinase was a major stumbling block in elucidating the mechanism of action, but eventually this was resolved. Acyclovir has become recognized as one of the safest drugs of all times with almost no adverse affects described during $2^1/_2$ decades of use (apart from those related to low aqueous solubility of the compound), including individuals who have used the compound for 20 years to suppress recurrent HSV. Acyclovir was the very first highly selective antiviral compound, and it was the prototype described as a 'second generation' nucleoside analogue. It eventually became available as an over-the-counter drug in the UK, an unthinkable development even a few years previously.

The prodrug strategy for enhancing oral bioavailability

The fact has remained that its low oral bioavailability gives acyclovir a pharmacological disadvantage. Several new analogues had been discovered to have similar antiviral properties in particular bromovinyldeoxyuridine (BVDU), synthesized by Phil Barr in the Laboratory of Stan Jones and Dick Walker at the Chemistry Department at the University of Birmingham (UK) and shown to be a potent inhibitor of HSV (type 1) and the related varicella-zoster virus (VZV) by Erik De Clercq at the Rega Institute of Medical Research (Belgium). BVDU is now on the market in Germany and other European countries for the treatment of herpes zoster (shingles). Acyclic guanosine analogues other than acyclovir were synthesized in several laboratories worldwide, one of those being ganciclovir (discovered by Julian Verheyden and John C. Martin then at Syntex) that later on would find a niche in the treatment of cytomegalovirus (CMV) infections in immunosuppressed patients.

BELOW:
A page out of Nick Oliver's notebook showing the chemical structure and first description in 1974 of the antiviral activity of acycloguanosine. The conclusion is 'very active; investigate further'.
IMAGE REPRODUCED WITH KIND PERMISSION OF GLAXOSMITHKLINE, NICK OLIVER AND PETER COLLINS



Another acyclic guanosine analogue, penciclovir, was developed in the laboratories of the former Beecham Pharmaceuticals company. Against HSV, penciclovir is comparable in activity and specificity with acyclovir. The realization that penciclovir has even poorer oral bioavailability than acyclovir resulted in a programme of medicinal chemistry led by Dr Mike Harnden which culminated in the synthesis of the molecule that was to become famciclovir. The key point here is this was the first antiviral orally available 'prodrug' (to be later marketed) and brought about a strategy that has been widely repeated for many other antiviral compounds. Famciclovir is rapidly absorbed when given orally and then converted to the active antiviral compound, penciclovir *in vivo*, following host enzymic conversion by two esterase steps and an oxidation step. In parallel work, Burroughs Wellcome developed several potential prodrugs of acyclovir and one of these was the valine ester of acyclovir which came to be known as 'valaciclovir' which is currently in widespread clinical use (for the same clinical indications as acyclovir). In fact, the first prodrugs to be described (back in 1983 by Hubert Vanderhaeghe and his colleagues at the Rega Institute) were the amino acid (glycine, alanine) esters of acyclovir, designed to make acyclovir more soluble in water. The prodrug strategy has now been widely adopted and the neuraminidase inhibitor produced by Gilead, oseltamivir, is one recent example (see above); valganciclovir, the valine ester of ganciclovir being another one.

HIV – a new virus threat

The science of antiviral research was well advanced when HIV/AIDS appeared as a major new virus disease in the early 1980s. The first effective antiviral compound (AZT, azidothymidine) was already among the library of compounds screened by Burroughs Wellcome and the National Cancer Institute (USA), and was promptly reported in 1985 to be a specific inhibitor of retroviruses, including HIV. The mechanism of action of AZT is based upon phosphorylation of the drug by cellular enzymes to AZT triphosphate, which then interacts at the substrate-binding site of the HIV reverse transcriptase, thereby acting as a chain terminator. The discovery of AZT was followed by several other dideoxynucleoside (ddN) analogues (ddI, ddC, d4T, 3TC, ABC, (–)FTC) so that at the time of writing seven ddN analogues, also referred to as NRTIs (i.e. nucleoside reverse transcriptase inhibitors) are formally licensed for the treatment of HIV infections. All these NRTIs act in a similar fashion: after their phosphorylation to the triphosphate, they interact as 'chain terminators' of the HIV reverse transcriptase, thus preventing the formation of the proviral DNA that otherwise would eternalize the infectious state (following integration of the proviral DNA into the host-cell genome).

In 1990 it came as a surprise that the HIV reverse transcriptase revealed a second target for interaction of HIV RT inhibitors, namely at an allosteric site, distinct from where the NRTIs interact. This site was originally dubbed the TIBO-binding site, as TIBO, together with HEPT analogues, were the first compounds found (by E. De Clercq and his colleagues) to interact in this way. Later, a succession of structurally different compounds, now termed NNRTIs (non-nucleoside reverse transcriptase inhibitors) were shown to interact in a manner similar to that of TIBO and HEPT, and of these NNRTIs, three, namely nevirapine, delavirdine and efavirenz, have been currently licensed for clinical use in the treatment of HIV infections.

The elucidation of the HIV genome revealed at an early stage the existence of the virus-specified protease and this was the declared target for several of the leading pharmaceutical companies. The team of chemists and molecular virologists at Roche led by Drs Joe Martin and Noel Roberts achieved the synthesis of saquinavir, the first peptide-based transition state mimetic. Saquinavir was shown to be active at nanomolar concentrations and was among the most potent antiviral substances yet described. Saquinavir was soon joined by several other similar compounds produced by competing companies. At present, seven protease inhibitors have been licensed for the treatment of HIV infections: saquinavir, ritonavir, indinavir, nelfinavir, amprenavir, lopinavir and atazanavir.

2003 witnessed the advent of the first 'HIV fusion' inhibitor, enfuvirtide (previously called 'T20'), which blocks viral entry by targeting the viral glycoprotein gp41 which is responsible for the fusion of the viral and cellular membranes. A problem with this compound is that, unlike all other anti-HIV drugs, which can be administered orally, enfuvirtide has to be given subcutaneously by injection twice daily.

It should be mentioned that virus drug resistance has not been a problem with herpesvirus chemotherapy (except in immunocompromised patients). However, resistance to antiviral drugs has emerged as one of the most important barriers to efficacy in the treatment of chronic infections, including HIV. In this case the key was to be found in history – the treatment of tuberculosis where cocktails of drugs were found to be necessary for the successful eradication of the mycobacteria over several months. There has been considerable opposition to the possibilities of drug combinations in the antiviral field (probably born from their inherent reputation for toxicity); therefore, the introduction and subsequent recognition of the value of drug combinations for the treatment of HIV infections were not instantaneous, but are now taken for granted.

The acyclic nucleoside phosphonates

The discovery in 1986 of HPMPA or (*S*)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine, by Antonin Holy and Erik De Clercq, heralded a totally new concept in the antiviral therapy era, that of the acyclic nucleoside phosphonates. These nucleotide analogues can be viewed as a kind of hybrid between acyclic nucleoside analogues (a class of molecules to which acyclovir, ganciclovir and penciclovir belong) and pyrophosphate analogues (phosphonoacetic acid and phosphonoformic acid), thus combining the assets of both approaches. In this sense HPMPA could be considered a hybrid of PAA (phosphonoacetic acid) with DHPA (2,3-dihydroxypropyladenine), a molecule discovered in 1978 by De Clercq and Holy as a broad-spectrum antiviral agent, but at that time overshadowed by acyclovir. As the first 'nucleotide' analogue, to be endowed with antiviral properties, HPMPA would subsequently give rise to numerous derivatives, three of which would eventually gain formal acceptance for the treatment of a wide variety of virus infections: cidofovir for the treatment of herpesvirus infections (CMV in AIDS patients), adefovir for the treatment of chronic hepatitis B and tenofovir for the treatment of HIV (AIDS), the latter two in the form of their oral prodrugs, adefovir dipivoxil and tenofovir disoproxil, respectively. Cidofovir, in addition to the indication for which it has been licensed (CMV retinitis in AIDS patients), also offers great potential for the treatment of papilloma-, adeno-, herpes- (other than CMV) and poxvirus infections (i.e. vaccinia, variola, monkeypox, molluscum contagiosum, orf). This is gratifying knowledge, as cidofovir may be useful in the prophylaxis and/or therapy of variola virus infections (smallpox) and complications (such as disseminated or progressive vaccinia) following vaccination with the smallpox vaccine vaccinia in immunocompromised patients.

Conclusions

Many decades after the birth of antibiotics, antivirals have, at last, definitely come of age – 37 antiviral drugs have been formally approved for the treatment of viral diseases. Their applications are primarily aimed at therapy of herpesvirus (HSV, VZV, CMV) as well as HIV, HBV, HCV and influenzavirus infections. Concomitantly with the availability of so many antiviral compounds, the genome sequences of many viruses have become available, and the structure and functions of many virus proteins known, thus defining novel specific targets for rational drug design. The difficulty of translating specific inhibitors into effective drugs remains a major task for the medicinal chemist and 'serendipity', which has aided virologists on several notable occasions in the past, will likely still have a role to play in the future.

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Further reading

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