Review

Progress in the clinical management of herpesvirus infections

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Summary

Antiviral drug discovery has produced a series of drugs active against herpesviruses in vitro. Several of these are now licensed and/or have been used in clinical practice. This article reviews the mechanisms of action of acyclovir, ganciclovir, penciclovir, sorivudine and foscarinet, the development of resistance to these drugs and their pharmacokinetic and cellular toxicities. Based upon the natural histories of HSV, VZV and CMV, treatment objectives for each virus are discussed and the performance of each drug matched against these objectives. Overall, it is concluded that the perfect drug for treating herpesviruses does not exist, but that significant progress has been made towards controlling several herpesvirus diseases. It is suggested that further progress will require not just improved drug discovery programmes, but also an understanding of different pathogeneses and an appreciation by practising physicians that antiviral drugs must be given early in the infectious process to achieve the best results.

Key-words: antiviral chemotherapy; natural history; pathogenesis; replication; treatment.

Introduction

Eight members of the Herpesviridae are now known to infect humans. During primary infection, herpesviruses establish latency, which allows the viral DNA to persist without expressing proteins that would be targets for an immune response. Intermittently, the latent genome can become activated to produce infectious virions. While the herpesviruses share aspects of molecular biology, replication, natural history and pathogenesis, they also differ extensively in important details. Historically, the herpesviruses have also been important in causing major diseases against which antiviral chemotherapy could be targeted, including the first successful demonstration of efficacy in a double-blind, placebo-controlled randomized trial (Whitley et al., 1977). The aim of this review is to consider what has been learned about clinical efficacy from the use of three nucleoside analogue antiviruses drugs and foscarinet which are licensed in the United Kingdom, and sorivudine, which is not licensed, but whose clinical trial data are informative. In total, these drugs have activity against herpes simplex virus type 1 (HSV-1), herpes simplex virus type 2 (HSV-2), varicella zoster virus (VZV) and cytomegalovirus (CMV), and the information gleaned may help in the design of clinical trials against the remaining four herpesviruses: Epstein-Barr virus (EBV), human herpesvirus type 6 (HHV-6), human herpesvirus type 7 (HHV-7) and the recently described AIDS-K3 herpesvirus (Chang et al., 1994).

Natural history of herpesvirus infections

Herpes simplex virus

The two serotypes of HSV (types 1 and 2) are differentiated in the laboratory by their possession of distinct neutralizing epitopes. Although the genomes of HSV-1 and HSV-2 are 50% homologous and both possess groups of genes which are highly conserved among herpesviruses, they have distinct characteristics (Roizman and Sears, 1993). Thus, HSV-1 is preferentially tropic for oral mucosa, while HSV-2 prefers genital mucosa. Although each virus can infect the alternative anatomical site, the rate of recurrence is significantly higher from the "preferred" mucosa (LaFerty et al., 1987).

Infection with one serotype may provide some cross-protection against the other serotype (Koelle et al., 1992). As a result of the obvious tendency of individuals to share viruses at the level of oral mucosa before they progress to transmission through sexual contact, HSV-1 is usually acquired before HSV-2. In individuals who escape HSV-1 infection, primary infection with HSV-2 can result in a clinically severe attack with complications. Even in those who have prior HSV-1 immunity, HSV-2 can present with severe symptoms, presumably reflecting acquisition of a greater than average viral inoculum.

From the perspective of genital herpes, the clinical correlates of these virological natural history data are initial
disease and recurrent disease. In the latter, disease results from reactivation of a latent infection in an individual with a history of genital herpes, i.e. is endogenous. In the former, the patient denies a previous episode of genital herpes, yet may have serological evidence of either primary or recurrent infection, i.e. the infection causing the genital herpes has been acquired exogenously. Note that many individuals must acquire infection from contacts who are unaware of their infectious nature (Mertz et al., 1992). Whether they are truly asymptomatic or merely unaware of the significance of minor genital symptoms is a moot point. Clearly, herpes simplex viruses are successful infectious agents because they do not readily disclose their presence and so can be transmitted by individuals who appear to be generally healthy. It is interesting that transmission from male to female appears to be more efficient than from female to male (Mertz et al., 1992).

From the perspective of oral herpes, the natural history is much simpler. Primary infection may produce stomatitis, although most infections are either mild or asymptomatic. Thereafter, reactivations occur to produce the familiar ‘cold sore’ on one anatomical portion of the lips. Individuals are infectious to others either while cold sores persist or at other times. A recent study (Tateishi et al., 1994) found asymptomatic HSV-1 shedding in 4.7% of 1000 patients attending an oral surgery department (approximately half detected by virus isolation and half by polymerase chain reaction, PCR), supporting the concept that asymptomatic excretion may be an important source of HSV-1 infection.

Herpes simplex encephalitis is almost always caused by HSV-1, except when it occurs in neonates who have acquired maternal HSV-2 during delivery. The disease follows primary infection in about one-third of cases where the brain is involved as part of the systemic infection. In the remaining two-thirds, it follows reactivation of latent virus (Whitley and Schlitt, 1991). Whether the virus establishes latency in all brains and only reactivates in a few, or whether peripheral reactivation occurs with transmission to the brain, is not known. The unusual bi-temporal distribution has been suggested to follow inoculation of virus into the nose, with replication and extension through the cribiform plate into the nerve endings of the first cranial nerves, which then connect to their respective temporal lobes (Dinn, 1980). In survivors of herpes encephalitis, histology in some cases shows gliosis and neuronal loss associated with persistent viral DNA which is detectable by PCR (Nicoll et al., 1993).

Varicella zoster virus

Chickenpox is a disease familiar to all clinicians and parents. The appearance of a generalized vesicular rash in a febrile child who is not particularly ill is characteristic. Each vesicle progresses through the pustule stage before scabbing, and complete healing occurs when the scabs fall off to reveal fresh epithelium. The onset of the rash corresponds to the time of secondary viraemia, which appears to be prolonged and to fluctuate so that successive crops of vesicles appear over a few days.

In a child with a large, initial viral inoculum, or one who is immunocompromised, the viraemia can seed internal organs with sufficient VZV to overcome local host defences. Under these circumstances, the child may develop pneumonitis and/or encephalopathy. It is not possible to identify individuals at risk of severe disease, but groups of people with normal underlying immunity who nevertheless have a generally increased risk of severity include: pregnant women (Brunelli, 1992), all other adults, adolescents (Balfour et al., 1992) and second- ary cases within families. The first three groups presumably represent the well-documented, but poorly explained, tendency for older individuals to have more severe forms of infectious diseases. The last group presumably reflects the greater viral inoculum transmitted when a child has frequent close contact with an infectious sibling than when he/she encounters the virus fleetingly.

Once a person has recovered from chickenpox, it appears clinically that VZV has been eradicated from the body, yet the virus has established latency in dorsal root ganglia. The ganglia that have latent VZV tend to be those which supply the dermatomes affected by the chickenpox rash. As a result, the areas affected by shingles mimic those affected by chickenpox, i.e. thoracic > cranial > others. It is not known what stimulates VZV to reactivate from the latent state. Most individuals have only one attack in their lifetime, although, if they live long enough, perhaps 60% of the population will ultimately experience shingles.

Shingles is a disease of the peripheral nervous system. The attack starts with a unilateral pain in any part of the body, which can be intense and may drive the patient to seek emergency medical attention. This pain can persist for 1–5 days and is very difficult to diagnose until the rash erupts. Once vesicles are seen spreading in a dermatomal distribution, the diagnosis should be clear. Healing of the rash occurs irrespective of the duration of the neural pain, which frequently becomes chronic, especially in the elderly. Motor involvement can occur, presumably as a result of infection of anterior horn cells, since the muscle groups are closely related to the affected dermatome. In AIDS patients, acute retinal necrosis has recently been described as a complication of zoster (Culbertson et al., 1986) which presumably follows VZV viraemia.

Cytomegalovirus

CMV has the most complex natural history of all the her-
pesviruses. Approximately 7% of neonates with congenital CMV are born with symptoms, while the remaining 93% are initially asymptomatic. Of this latter group, approximately 15% will progress to develop symptoms over the next 5 years. Symptoms are referable to the two major target organs of the brain and the inner ear, producing neurological dysfunction and/or sensorineural hearing loss. In 20% of children born with symptoms, the disease process is so severe that they die during the first year of life (reviewed in Fowler et al., 1992).

The sites of latency of CMV are not known. The virus can be detected in saliva, urine, blood and semen. It is transmitted by all organ allografts, including blood, and can be found at autopsy in most tissues of the body. CMV thus has the ability to replicate in many, if not all, cell types in vivo, in stark contrast to its restricted growth in fibroblasts in vitro. Presumably, cell cultures of the differentiated cells normally permissive for CMV cannot yet be propagated in vitro. Given the broad range of cells infected in vivo it is questionable whether CMV has a single site of latency. Studies with PCR of blood cells have detected CMV in monocytes/macrophages (Taylor-Wiedeman et al., 1991) rather than B or T lymphocytes, but it is not clear if this represents latent infection or low-level active infection. The situation is quite distinct in the immunocompromised host where CMV is frequently detected in polymorphs and also endothelial cells circulating in the peripheral blood (Greffe et al., 1993).

In common with HSV and VZV, reactivation in the recipient can lead to infection following transplantation. In addition, CMV can be transmitted with the donor organ or through blood products. Overall, approximately 50% of transplant patients excrete CMV at some stage after transplantation. Any subgroup of patients can develop CMV disease but, in recipients of solid organs, there is a preponderance of disease among those whose donor was seropositive (Paya et al., 1993). This is circumstantial evidence of transmission with the donated organ and molecular biological techniques have been used to prove that the donor can indeed be a source of virus for individuals who are seronegative (Wertheim et al., 1983) or seropositive (Grundy et al., 1988) before transplantation. In contrast, in bone marrow transplant (BMT) patients, the major source of CMV disease is reactivation of virus in the recipient. Again, this has been proven by molecular biological techniques (Winston et al., 1985). When the donor marrow is depleted of T cells, there is evidence for adoptive transfer of humoral immunity into the recipient (Grob et al., 1987) so that donor immunization before transplant represents a potential approach to controlling CMV disease (Wenigeris et al., 1986). More recently, it has been shown that cytotoxic T cells of donor origin can be expanded in vitro and transferred into the recipient (Ridell et al., 1992).

CMV excretion begins in the first month following transplantation and rises to high levels during the second and third months, when it typically is associated with disease. In some cases, CMV disease can present, like VZV, many months after transplantation. Excretion of CMV is often asymptomatic or is accompanied by mild febrile episodes. This may develop into a septicaemia-like picture, with prostration, hypotension and spiking fever. During this viraemic phase, CMV may spread to the lungs to cause interstitial pneumonitis, to the liver to cause hepatitis or to the gastrointestinal tract to cause diarrhoea and/or abdominal pain. CMV infection can also involve the eye, causing retinitis, although this is uncommon and usually occurs many months after transplant. In all of these cases, the pathogenesis of CMV disease is predominantly lytic, i.e. cells are destroyed by virus replication; in contrast, pneumonitis appears to be immunopathologically mediated (Grundy et al., 1987).

In AIDS patients the same general principles apply in that CMV infection is increasingly likely as the patient becomes progressively immunocompromised (Gallant et al., 1992). However, the range of diseases which result is much broader. Retinitis is the major CMV disease in AIDS patients, in contrast to its rarity in transplant patients. Gastrointestinal involvement accounts for approximately 10% of CMV disease in AIDS patients (Diekerich and Rahmin 1991). Increasingly, neurological disease associated with CMV is being recognized. Clinical presentations include encephalitis, polyradiculopathy and painful peripheral neuropathy. At autopsy, CMV infection can be found in multiple organs (Pillay et al., 1993b) so the range and extent of CMV diseases may be greater than appreciated clinically.

**Overview of herpesvirus replication**

Reactivation of herpesviruses from latency requires the information in the viral genome to be capable of active expression (i.e. the latent genome must not be defective) and for essential enzymes and precursor molecules to be supplied by the cell. Given the large DNA genomes of herpesviruses (150–229 kbp), the availability of nucleotide triphosphates is rate-limiting for reactivation from latency.

The genomes of HSV and VZV are latent in neurons which are terminally differentiated and therefore unable to divide, and so nucleotide triphosphates are not found in high concentrations. To facilitate successful reactivation in such cells, herpesviruses have evolved to encode enzymes which can increase the availability of the precursor molecules essential for a DNA virus (Roizman and Sears, 1993). Such enzymes include ribonucleotide reductase (which scavenges RNA into DNA) and thymi-
dine kinase (TK, which phosphorylates many nucleosides as well as thymidine to their monophosphates). While several enzymes represent potential targets for future antiviral chemotherapy, it is the virus-encoded TK that is important for contemporary drugs. The viral TK recognizes a broader range of substrates than the analogous enzyme encoded by the host and, as a result, drugs can be targeted for activation by the virus-encoded TK which would not be activated in uninfected cells. The net result is that, upon entering a cell harbouring a herpesvirus genome, the antiviral drug is phosphorylated to its monophosphate form. Cellular enzymes then phosphorylate it further to the active triphosphate. In some cases TK is responsible for the second phosphorylation, for example the conversion of thymidine monophosphate to the diphosphate, and the same is true for some nucleoside analogue drugs discussed below. This enzymic activity is termed thymidylylate kinase. CMV does not possess a TK but has a gene termed UL97, which phosphorylates ganciclovir and acyclovir to their monophosphates (Littler et al., 1992; Sullivan et al., 1992).

Antiviral mode of action

The five drugs to be considered are acyclovir, ganciclovir, penciclovir, sorivudine and foscarnet (see Fig. 1 for structures). The first four drugs must be effective substrates for the herpesvirus monophosphorylating enzyme, and Table 1 shows their relative efficacies. The best substrates are sorivudine for VZV, ganciclovir for HSV-2 and sorivudine or penciclovir for HSV-1. Acyclovir, the mainline drug used in clinical practice for all of these viruses, is a relatively poor substrate.

The monophosphate must be activated to the triphosphate to inhibit DNA polymerase. For acyclovir, penciclovir and ganciclovir (Mathews and Boehme, 1988) this is accomplished by several cellular enzymes, including guanylate kinase (Miller and Miller, 1980) and phosphoglycerate kinase (Miller and Miller, 1982). Sorivudine monophosphate is converted to the diphosphate by the

| Table 1. $K_m$ of various drugs against three herpesvirus-encoded phosphorylating enzymes |
|---------------------------------|-------|-------|-------|-------|
| Thymidine kinase                | Acyclovir | Penciclovir | Ganciclovir | Sorivudine |
| HSV-1                           | 100    | 1.5    | 10     | 0.9    |
| HSV-2                           | 90     | ND     | 20     | 55     |
| VZV                             | 830    | ND     | 1300   | 0.03   |

ND, not determined.

Table 2. $K_m$ of various drug triphosphates against four herpesvirus-encoded DNA polymerases

<table>
<thead>
<tr>
<th>DNA polymerase</th>
<th>Acyclovir</th>
<th>Penciclovir</th>
<th>Ganciclovir</th>
<th>Sorivudine</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV-1</td>
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<td>8.5</td>
<td>0.06</td>
<td>ND</td>
</tr>
<tr>
<td>HSV-2</td>
<td>0.03</td>
<td>6.1</td>
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<td>1.6</td>
<td>ND</td>
<td>0.34</td>
</tr>
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<td>CMV</td>
<td>0.008</td>
<td>ND</td>
<td>0.022</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND, not determined.

1Fyfe et al., 1983; 2Vere Hodge, 1993; 3Cheng et al., 1981; 4Ellis et al., 1987; 5Roberts et al., 1993.

Thymidylylate kinase activity of VZV and then to the triphosphate by cellular enzymes. HSV-1 TK, but not HSV-2, can also convert sorivudine monophosphate to the diphosphate (Yokota et al., 1989).

The relative abilities of the drug triphosphates to inhibit DNA polymerase enzymes are shown in Table 2. Acyclovir triphosphate is now seen to be the best inhibitor of each enzyme.

Thus, when reviewing the biochemical basis for clinical efficacy against each herpesvirus, information about the phosphorylation and the potency of inhibition by the triphosphate must be considered as a whole. In addition, each drug has other distinctive characteristics which are discussed below.

Acyclovir

As the DNA chain begins to elongate, acyclovir triphosphate is attached, a process catalysed by the viral DNA polymerase (McGuirt and Furman, 1982; Reardon and Spector, 1989). When the DNA polymerase subsequently attempts to add the next required nucleotide to the growing DNA chain, it is unable to complete the reaction because acyclovir lacks the free hydroxyl group required. Because its attachment to the DNA chain prevents further nucleotide incorporation and therefore DNA chain elongation, acyclovir is referred to as an obligate chain terminator. In addition, the enzyme is unable to separate from the complex to catalyse further reactions so that acyclovir acts as a suicide inhibitor (Furman et al., 1984).

Ganciclovir

Ganciclovir is active in vitro against a wider range of herpesviruses than acyclovir, but the considerable side-effects have limited clinical studies to the assessment of efficacy in patients with CMV infections. The UL97 phosphotransferase of CMV activates ganciclovir to the monophosphate (Littler et al., 1992; Sullivan et al., 1992).
Fig. 1. Chemical structures of drugs discussed in the article.
The triphosphate inhibits the viral DNA polymerase and is incorporated into viral DNA with the second hydroxyl on the compound allowing some chain extension prior to eventual termination of viral DNA elongation, so ganciclovir is not an obligate chain terminator.

**Penciclovir**

Although the structure of penciclovir is closely related to that of ganciclovir, differing only in the presence of an oxygen in the acyclic side-chain (Fig. 1), its initial activation by the herpesvirus-encoded TK resembles that of acyclovir. Penciclovir is phosphorylated more efficiently than acyclovir and its triphosphate has a significantly longer half-life, resulting in relatively high concentrations of penciclovir triphosphate in the infected cell (Vere Hodge and Perkins, 1989).

Penciclovir triphosphate inhibits the viral DNA polymerase through competition with deoxyguanosine triphosphate and is incorporated into the viral DNA. However, like ganciclovir, penciclovir is not an obligate chain terminator as DNA chain extension can be detected after penciclovir triphosphate incorporation (Earnshaw et al., 1992; Vere Hodge, 1993).

**Sorivudine**

Sorivudine (BV-ara-U) is a thymidine nucleoside analogue (Fig. 1) which has potent in vitro antiviral activity against VZV- and HSV-1-infected cells (Machida and Watanabe, 1991). The triphosphate inhibits viral DNA synthesis but is not an obligate chain terminator (Yokota et al., 1989).

**Foscarnet**

Foscarnet is structurally unrelated to the other drugs discussed here. It is a pyrophosphate analogue which inhibits herpesvirus DNA polymerases by interacting with the pyrophosphate binding site. Selectivity is achieved because the drug has less activity against host cell DNA polymerases. Foscarnet is not phosphorylated or activated by a herpesvirus-encoded enzyme; the drug is administered intravenously in its active form.

**Resistance**

The potent inhibition of virus replication exhibited by these drugs produces a strong selective pressure for the emergence of resistant strains. The genetic loci subject to this pressure encode the phosphorylating enzyme and the DNA polymerase.

**Varicella zoster virus**

Much less is known about acyclovir resistance in VZV although the general principles outlined above apply. The first case of TK- VZV was described by Linnemann et al., (1990). An AIDS patient received multiple courses of acyclovir for the treatment of shingles, as well as low-dose oral acyclovir. Initial isolates were sensitive to acyclovir but later isolates were TK- and resistant. Interestingly, the TK- strain was associated with persistence of zoster lesions, but not with the formation of new lesions, which is consistent with the proposed decreased replicative ability and virulence of TK- strains.
Biochemical studies of one resistant strain (Roberts et al., 1991) have shown that a single point mutation in the TK gene can dramatically decrease acyclovir phosphorylation whilst maintaining the ability to phosphorylate thymidine (TK\(^\text{\textsuperscript{a}}\) phenotype). Subsequently, a series of point mutations in different regions of TK have been described (Talarico et al., 1993), together with mutations which lead to premature termination (TK\(^\text{\textsuperscript{c}}\) phenotype).

**Cytomegalovirus**

Again, the general principles outlined for HSV apply to CMV. Indeed, it was through marker rescue experiments using DNA from a ganciclovir-resistant strain selected in vitro that gene UL97 was shown to be important for phosphorylating ganciclovir (Litter et al., 1992; Sullivan et al., 1992). The original resistant strain described had a 12-nt deletion involving the putative active site of the UL97 phosphotransferase. This change has so far not been seen in clinical practice, although other changes have been mapped to this region. Two different changes have so far been shown to be capable of inducing phenotypic resistance of CMV to ganciclovir. The first is at position 595 (Wolf et al., 1995) which is immediately adjacent to the 12-nt deletion originally described. The wild-type leucine at this position can be mutated to phenylalanine or serine (Wolf et al., 1995). The second change is at position 460 where methionine can be changed to isoleucine (Lurain et al., 1994).

**Pharmacokinetics**

Acyclovir has limited bioavailability and the proportion absorbed decreases as the dose is increased. There is thus a practical limit to the plasma levels that can be achieved orally. Acyclovir is excreted largely unchanged in the urine. Enhanced acyclovir bioavailability is provided by the L-valyl ester, valaciclovir, which is cleaved to release acyclovir in the gut and liver by an enzyme referred to as valaciclovir hydrolase. Little valaciclovir is detected in the plasma. Multiple oral doses of valaciclovir (≥1000 mg four times daily) result in plasma acyclovir concentrations similar to those achieved with intravenous acyclovir (5 or 10 mg kg\(^{-1}\) three times daily) but without the sharp peak concentrations.

Ganciclovir is very poorly bioavailable so most studies have relied upon intravenous dosing. Recent studies report that oral ganciclovir (2000 mg three times daily) can produce sufficient absorption to allow maintenance of CMV reinitis in AIDS patients. Ganciclovir is excreted largely unchanged in the urine.

Penciclovir is very poorly bioavailable. The prodrug famciclovir has 77% bioavailability and is rapidly converted to penciclovir in gut and liver. One ester is preferentially removed, then the second, followed by oxidation by aldehyde oxidase. Little famciclovir is detected in plasma. Penciclovir is excreted largely unchanged in the urine.

Sorivudine has good oral bioavailability. In mice the drug is metabolically stable, but in humans the major metabolite is bromovinylnuracil (BVU) and 2% of the drug is excreted in this form in the urine (Ashida et al., 1993). Unfortunately, this metabolite inhibits the enzyme dihydrooridine dehydrogenase which is normally responsible for degrading drugs such as 5-fluouracil. In cancer patients in Japan, this drug interaction led to a catastrophic accumulation of 5-fluouracil producing fatal bone marrow suppression (Meeting Report, 1994).

Foscarnet is not orally bioavailable and is administered by intravenous infusion. It is excreted largely unchanged in the urine.

**Cellular toxicity**

When designing an antiherpes drug a desired characteristic is that it is activated by a virus-encoded enzyme to promote selectivity. Ideally, the activated compound should then inhibit a virus-coded activity to further promote selectivity. However, this is not an absolute requirement because cells triggered from latency to productive infection are already destined to die as a result of the herpesvirus infection. The active form of the drug could exhibit cellular toxicity yet still have an unblemished safety profile provided that it was only activated in virus-infected cells. Clearly, if the active form of the drug has no cellular toxicity, this is an additional advantage and such lack of cellular toxicity is particularly important if the selective action of the drug is not absolute. Given the vast number of cells in the body which are not infected with a herpesvirus during an infection, a small risk of toxicity to a large number of cells may outweigh the clinical benefit seen in the smaller number of virus-infected cells. Furthermore, lack of toxicity to uninfected cells is particularly important if the drug is to be used chronically.

In addition to showing selectivity at the monophosphorylation stage, the nucleoside analogues discussed here show preferential inhibition of the viral rather than cellular DNA polymerase once converted to their triphosphate forms. Thus, acyclovir triphosphate has a 10–30-fold greater affinity for viral DNA polymerases than for their cellular counterparts (Ellin et al., 1977). Ganciclovir also inhibits the cellular DNA polymerase, although at a higher concentration than that required for inhibition of the viral DNA polymerase. Penciclovir triphosphate has low affinity for DNA polymerase α.

The possibility of cellular toxicity is evaluated extensively in formal studies of cells and animals before pro-
ceeding to trials in humans. In the following sections, information about potential cellular toxicities of each of the four licensed drugs is reviewed.

To enable comparisons to be made between the four drugs, the data provided in the USA product data sheets as mandated by the regulations of the Food and Drug Administration have been quoted. Where given, figures in brackets provide an estimate of the equivalent dose exposure in humans using the parameter of area under the curve of plasma concentration × time.

In vitro mutagenesis

Multiple screening assays are used to detect genotoxic potential, such as genetic mutations in S. typhi or E. coli and unscheduled DNA synthesis in mammalian cells. Mouse lymphoma and human lymphoma assays are used to detect chromosomal aberrations.

Acyclovir was not mutagenic in standard microbial assays. At high dosage, some chromosomal breaks were seen in vitro, but were not seen in humans. Acyclovir was clastogenic in Chinese hamsters (at 380 × human exposure). Cell transformation was seen in one of two assays at high dosage (31–63 ×). Mutagenicity was seen at high dosage (250 ×) in a mouse lymphoma assay.

Ganciclovir was not mutagenic in the standard Ames test. It was mutagenic in a mouse lymphoma assay and clastogenic in a mouse micronucleus assay (2.8–10 ×).

Famciclovir and penciclovir were not mutagenic in standard microbial assays. Famciclovir and penciclovir caused chromosomal aberrations in human lymphocytes. Penciclovir, but not famciclovir, produced gene mutations/chromosome aberrations in a mouse lymphoma assay.

Foscarnet was genotoxic to BALB/3T3 cells and produced chromosomal aberrations in a sister chromatid exchange assay. Increases in micronucleated polychromatic erythrocytes were seen in mice at doses used in humans.

Animal carcinogenicity

Acyclovir had no carcinogenic effect in rats or mice given life-time exposures of 3–6 × (mouse) and 1–2 × (rat) estimated human exposure.

Ganciclovir is carcinogenic in mice at low concentrations (0.1–1.4 ×). In males, tumours of preputial gland and forestomach, and in females tumours of forestomach, ovaries, uterus, mammary gland, ciliary gland and liver, were seen. The no-carcinogenic-effect dose was 0.01 × estimated human exposure.

In 2-year dosing studies, famciclovir produced a significant increase in mammary adenocarcinomas and marginal increases in fibrosarcomas or squamous cell carcinomas of the skin in female rats receiving the largest tested dose, but not in those receiving smaller doses. No such effects were seen in male rats, although male mice showed increases in fibrosarcomas/squamous cell carcinomas similar to those seen in female rats. The largest dose in female rats was equivalent to 1.5 × the human systemic exposure at a USA recommended dose of 500 mg three times daily (note that 250 mg three times daily is the dose recommended in the UK). The equivalent exposure for male mice was 0.4 × human systemic exposure.

Foscarnet showed no evidence of carcinogenicity in rats and mice but the studies were limited by poor oral bioavailability so that doses approximating human exposure have not been studied.

Impaired fertility

Acyclovir causes testicular atrophy in rats at very high doses (24–48 ×) and aspermatogenesis in dogs (47–177 × human doses). A study in humans did not show evidence of impaired sperm production in patients receiving acyclovir (Douglas et al., 1989). Acyclovir had no effect on fertility or reproduction in rats or mice.

Ganciclovir decreases fertility in female mice and increases embryo lethality at 1.7 × human exposure. Changes seen in male offspring included hypoplasia of the testis and seminal vesicles. In male mice and dogs, spermatogenesis is impaired at low dosages (0.03–0.1 ×).

Famciclovir had no effect on fertility in female rats. In male rats, mice and dogs, testicular toxicity included atrophy of seminiferous tubules, decreased sperm count and/or sperm with abnormal morphology or reduced motility. Toxicity increased with chronic administration and high doses. The no-observable-effect dose in rats after 26 weeks was equivalent to 0.2 × the human exposure at 500 mg three times daily. Testicular toxicity was also seen following chronic administration to mice (0.4 ×) and dogs (1.7 ×).

Foscarnet had no effect on fertility in rats, but only low drug concentrations could be studied. A slight increase in skeletal abnormalities in rats has been described.

Teratogenicity

Acyclovir is not teratogenic in rats, mice or rabbits in standard tests, in a non-standard test in rats, head and tail abnormalities and maternal toxicity were seen at high doses (63–125 ×).

Ganciclovir is embryotoxic to rabbits and mice. It causes fetal resorption at $2 \times$ estimated human exposures. Teratogenic effects seen in rabbits included cleft palate, microphthalmia, aplastic kidney and pancreas, hydrocephaly and brachygnathia ($2 \times$ estimated human exposure).

Famiclovir produced no adverse effects on embryofetal development in rats and rabbits.

Foscarnet given subcutaneously to female rats or rabbits produced a slight increase in skeletal abnormalities. The doses used equated to one-eighth of the estimated human exposure in the rat and one-third in the rabbit.

Breast feeding

Acyclovir is excreted in human breast milk. Penciclovir and foscarnet are excreted in rat breast milk at concentrations higher than those in plasma.

Treatment objectives

*Herpes simplex virus*

Genital herpes. For genital herpes, controlled clinical trials have shown that acyclovir can decrease the time required for patients to become culture-negative, decrease the time to complete healing, and reduce the incidence of complications which are particularly distressing (Bryson et al., 1983; Mertz et al., 1984).

Treatment of the acute episode has no effect on the future rate of recurrences because HSV DNA remains latent in sensory nerve ganglia from where it can reactivate on multiple occasions. In future, it may be possible to use ribozymes to target this DNA and destroy its integrity in situ. At present, the disease must be controlled by using antiviral drugs to prevent activation of the latent genome. Thus, the chronic administration of acyclovir has been shown to be effective in suppressing reactivation of genital herpes (Douglas et al., 1984; Straus et al., 1984) and represents a major advance in controlling recurrent genital HSV infection (Goldberg et al., 1993). In my opinion, the treatment benefit of such prophylaxis is far greater than that seen when each individual episode of reactivation is treated with the same drug.

Nevertheless, there remains the possibility that prophylaxis could allow disease to be suppressed but that infection could continue unhindered, with possible transmission of HSV to others. Some patients do experience occasional breakthrough recurrences and should be advised that they remain infectious to others at these times (Goldberg et al., 1993). Is it possible that recurrences could occur asymptotically and be transmissible to others? In general, transmission is facilitated by a large virus inoculum so that small breakthrough reacti-ations occurring in the context of suppressive antiviral chemotherapy may not represent a major public health problem. Nevertheless, this potential for transmission is under active investigation and definitive data are awaited using PCR assays as well as virus isolation. When interpreting the results of such studies, we should remember that other interventions available to the patient, such as condom usage, may not provide 100% protection. A comparative study of acyclovir prophylaxis vs. universal condom usage would provide useful data, although the study could obviously not be placebo-controlled.

For oral herpes, a major treatment objective is to reduce the time to complete healing. Oral acyclovir can speed healing (Raborn et al., 1987). Several double-blind, placebo-controlled trials have been performed with topical acyclovir. In summary, 5% acyclovir in an ointment base had no clinical benefit (Sprouse et al., 1982; Raborn et al., 1989), whereas in some (Fiddian et al., 1983; Van Vliet et al., 1983), but not all (Shaw et al., 1985) studies it was reported that 5% acyclovir in a cream base had a modest effect on the parameters of healing.

For prophylaxis of oral herpes, several interventions have been reported to be effective, including oral acyclovir given 400 mg twice a day to those about to begin a skiing holiday (Sprouse et al., 1988), and sun-screen applied to individuals subjected to experimental ultra-violet irradiation (Rooney et al., 1991).

While all of these results are consistent with the idea that acyclovir can improve the healing of cold sores, the overall clinical benefit is not dramatic. Indeed, one can question whether the use of such a successful antiviral drug for the treatment of a condition which many believe to be trivial can be justified on medical grounds. Nevertheless, patients with cold sores will undoubtedly request treatment for psychological and cosmetic reasons which they perceive to be important. As with genital herpes, the best results seem to be obtained when the drug is given prophylactically rather than for acute treatment, but it would be difficult to justify chronic administration of such a drug under normal circumstances.

*Herpes simplex encephalitis.* This condition can be devastating and was frequently fatal before therapy became available. HSV encephalitis is important historically because it was the first condition for which systemic antiviral chemotherapy (vidarabine) was shown to be superior to placebo in a controlled clinical trial (Whitley et al., 1977). Subsequently, acyclovir was shown to be superior to vidarabine (Whitley et al., 1986). In neonates with HSV-2 encephalitis, the drugs have similar efficacies although acyclovir is easier to administer (Whitley et al., 1991).
The ideal drug for HSV treatment. The desired characteristics of the ideal drug for treating HSV infections are shown in Table 3. Controlled clinical trials have shown that acyclovir has most of these characteristics and so is clinically the treatment of choice. This does not mean that acyclovir has removed the scourge of HSV infections from the world; however, the drug does not affect latent HSV, so individuals are still at risk of future disease once a treatment course has been completed. Nevertheless, acyclovir has dramatically improved the outlook for individuals with HSV infection and, historically, has proved the concept that antiviral drugs can be used safely in routine clinical practice. The best results are obtained when the patient and physician both understand the natural history of HSV infections and administer acyclovir early during the course of infection. For genital herpes the best treatment strategy seems to be the use of long-term oral acyclovir to keep the virus suppressed into latency, which has greatly improved the quality of life for patients whose infections would otherwise recur frequently.

For HSV encephalitis, there is significant room for improvement as many patients are still left with neurological damage. Much of this poor clinical outcome results from a delay in diagnosis, so that the antiviral drug is not administered promptly enough. In my opinion, this is the area that requires most attention; clinicians should be educated to begin acyclovir therapy early in the course of acute neurological disease, which may or may not turn out to be herpes encephalitis. While the search for drugs with even greater activity against HSV and with even better penetration into brain tissue should continue in research laboratories, no antiviral drug can be expected to provide benefit until it is given to the patient. If the treatment of HSV encephalitis is delayed, significant loss of vital neurons will have occurred and no antiviral drug can be expected to repair dead nerve cells. Nevertheless, newer drugs such as valaciclovir should be tested in controlled trials to determine if chronic administration after treatment of the acute encephalitic illness can provide additional benefit.

Following the broad success of acyclovir it will be difficult to identify better drugs and to demonstrate their superiority as very large clinical trials will be required. Although the bioavailability of acyclovir is relatively low, absorption is sufficiently good to permit control of all HSV diseases. Thus, the achievement of higher bioavailability through the use of valaciclovir is unlikely, in my opinion, to offer major therapeutic benefits for genital herpes, although it may provide some patient benefit in terms of dosage frequency. Valaciclovir may also prove superior for its effects in those patients who have breakthroughs despite acyclovir prophylaxis, and for its effect on asymptomatic shedding, as any chance to control further the cycle of transmission of infection is to be welcomed. Ganciclovir is generally acknowledged to be too toxic for the treatment of HSV infections; however, if it is prescribed for CMV disease it may well provide cover for HSV outside the central nervous system, although this has not been proved in controlled clinical trials. Similarly, the toxicity profile of famciclovir, particularly animal carcinogenicity, does not appear propitious for suppression of genital herpes. Sorivudine is active against HSV-1 but the difficulty of distinguishing this from HSV-2 clinically mitigates against its use. In theory, sorivudine could be considered for labial herpes but current concerns about its toxicity profile make this unlikely.

Varicella zoster virus

Chickenpox. Placebo-controlled trials of acyclovir have shown that new lesion formation and the rate of lesion healing can be improved (Balfour et al., 1990, 1992; Dunke et al., 1991; Wallace et al., 1992). Insufficient cases of complications were observed to provide definitive data, but the reduction in the proportion of individuals with active vesicle formation after day 3 of treatment is encouraging.

One might imagine that the discovery of a safe drug that is active against a major childhood illness which cannot at present be prevented by immunization would be greeted with acclamation. However, medical communities have become more refractory to medical advances and have come to question the cost:benefit advantages of each new treatment. For acyclovir the treatment of a case of chickenpox will, typically, turn a 5-day illness into a 4-day illness. Although an argument can be made that this provides a financial advantage to parents who can return to work 1 day earlier than expected, it must be remembered that this financial benefit does not accrue to the budget incurring the extra financial costs for acyclovir. On
balance, therefore, medical groups charged with reviewing these data have indicated that the median benefit of 1 day’s earlier healing is not worth the additional financial cost. An alternative interpretation of the data is that by 4 days (i.e. day 3 of treatment) the chickenpox illness will be in the recovery phase in virtually all children who have received acyclovir, compared with 80% for controls. These data indicate that acyclovir can reduce severity in those destined to have above-average chickenpox severity and suggest that future trials are required to demonstrate whether acyclovir can reduce the incidence of complications as a result of chickenpox. If this were to be confirmed, then the widespread use of acyclovir would be indicated because approximately 100 children die each year in the USA alone from chickenpox (Preblud, 1986).

Until such a trial is reported, clinicians may wish to use acyclovir in selected patients who would otherwise have a high risk of severe disease and death. I would recommend treatment of all pregnant women and all other adults. A case could be made for treatment of all adolescents, since they have a higher than average risk of severe disease. I do not feel at present that a case has been made to justify prescription of acyclovir for all children with chickenpox, in whom the disease is relatively benign.

Shingles. For clinical investigators, the presence of the skin rash of herpes zoster is a boon, because it allows the effects of antiviral drugs to be visualized directly. Unfortunately, this ready availability has, I believe, distracted investigators from studying the effects of antiviral drugs on the real clinical target, the nerves. For example, there is good agreement among investigators for the use of parameters such as time to new vesicle formation, time to crusting and time to complete healing as measures of antiviral efficacy. There is no consensus, however, about the way to measure the neural pain of shingles, and so no baseline against which the efficacy of antiviral drugs can be assessed. Some workers describe the pain associated with the dermal rash (acute pain) and differentiate this from the neural pain which persists after the rash has healed (post-herpetic neuralgia). This division implies that the neural damage and pain develop late in the clinical course, whereas the pathological processes are almost certainly set in train before the rash appears. The division of pain times is also difficult to calculate because the duration of the rash varies in different people; a cut-off of 30 days after onset of rash is often used as a compromise.

Overall, the term ‘zoster-associated pain’ is preferred, which records pain as a continuum and allows time-to-event survival analyses to be performed (Wood, 1993). The shape of these curves indicates the times at which pain is being affected. Indeed, the time analyses should ideally be set to zero when pain is first experienced, rather than the date of onset of the rash, to emphasize the critical importance of controlling pain at all phases of the disease. If this is done, it will be seen that antiviral chemotherapy administered within 24 h of rash onset is in reality being given 2–6 days after the patient first experiences pain and so, presumably, 2–6 days after VZV replication began.

Irrespective of the type of antiviral drug to be given, it is essential to encourage clinicians to recognize shingles before the rash appears, difficult though this is. Unlike the case of recurrent HSV, the patient cannot help readily as he/she is likely to have experienced an attack previously, and so is unaware of the natural history and significance of prodromal symptoms.

The most important treatment objective is to reduce neural damage, but as the precise pathogenesis is not known, this can only be measured by the chronic persistence of pain. Whether acyclovir has an effect on zoster-associated pain has been a controversial issue. A meta-analysis of studies that followed all patients for at least 6 months has shown a significant reduction in total pain scores, both in the initial phase and in the post-herpetic phase (Crooks et al., 1991). Thus, it is reasonable to conclude that acyclovir, given within 72 h of rash onset, can decrease the incidence of zoster-associated pain, but that there is still room for substantial further improvement.

Recent studies have indicated that famciclovir (Degreef et al., 1994) and valaciclovir (Beutner et al., 1995) can also significantly reduce zoster-associated pain when compared with placebo. A comparison between famciclovir and acyclovir suggested superiority of the former, based upon analyses of only a subgroup of patients (Degreef et al., 1994). In my opinion, this conclusion is premature and should be tested in a larger group of patients with analysis of all patients according to predetermined analytical parameters. Famciclovir has a potential advantage in terms of the long intracellular half-life of penciclovir triphosphate, yet this has a lower affinity for VZV DNA polymerase than acyclovir triphosphate. It is therefore not clear from these in vitro parameters which drug looks more promising and only a large comparative controlled trial can settle the issue. Such a trial has clearly demonstrated the superiority of valaciclovir over acyclovir, so a comparative study of famciclovir vs. valaciclovir is warranted. While the details of such studies are planned, elderly patients at risk of chronic zoster-associated pain can be reassured that considerable efforts are being made to identify drugs active against this debilitating disease and to make them readily available. While waiting for the results of such comparative trials, I believe we should be directing our attention towards making sure that all patients who could benefit from these antiviral
drugs, as well as their primary care physicians, are aware that treatment is available and should be started early.

Another important objective in the treatment of shingles is to reduce the complications associated with the disease. In the immunocompetent, these are largely restricted to the eye and are frequently serious. Many parts of the eye can be involved when VZV reactivates on the face, particularly, but not exclusively, in the ophthalmic division of the trigeminal nerve. VZV infection may damage the corneal epithelium directly or indirectly by causing scarring of the tarsal margins leading to trauma. Involvement of the internal structures of the eye can cause uveitis, which is probably immunopathologically mediated. Overall, approximately 50% of patients with ophthalmic zoster will develop eye complications (Harding, 1993). Once established, the treatment of these complications is difficult and the outcome is often unsatisfactory. The best method of treatment is prevention, so all patients with zoster involving the face must be treated promptly and should be referred directly to an ophthalmologist for assessment.

Many patients with shingles describe loss of body image, especially if the rash involves the face. This can be serious and can precipitate depression. Clearly, an antiviral drug which would halt replication rapidly and promote skin healing without scarring is required.

The ideal drug for VZV treatment. The desired characteristics of an ideal drug for VZV infections listed in Table 3 show that there are different safety requirements for the treatment of VZV and HSV. Thus, for VZV, safety in chronic administration is not as strong a requirement unless attempts are to be made in the future to suppress shingles in the immunocompromised. Instead, the ideal drug would be used for short courses only, but must be safe in the elderly and in children to allow treatment of shingles and chickenpox, respectively. Because of differing metabolic handling of drugs in people at the extremes of age, together with differences induced by co-existing disease of the kidneys and/or liver in the elderly, this means that short-term safety is the prime concern for VZV therapy.

Studies have shown that acyclovir can meet all of these criteria, including safety, and so has been called the 'gold standard' against which newer drugs must be compared. There is still room for improvement on the characteristics of acyclovir, however, particularly in relation to areas of inherent activity against VZV and in bioavailability. It is hoped that valaciclovir, which is expected to have a safety profile identical to that of acyclovir and an improved bioavailability, will improve treatment in these areas. Likewise, famciclovir has better bioavailability and has been reported to be superior to placebo for the treatment of shingles. The results of a large-scale direct comparison of efficacy and safety between famciclovir and valaciclovir for the treatment of zoster are awaited. The significance for humans of the famciclovir rodent carcinogenicity data are not clear. Since the effect is related to both dose and duration, short-term trials in elderly patients with zoster can be justified. However, the same may not be true for chickenpox since exposure of young individuals would be required.

The ED<sub>50</sub> of sorivudine against VZV is 0.001 μM compared with 14 μM for acyclovir (Shigeta et al., 1993). Sorivudine is currently undergoing clinical trials in the USA for the treatment of shingles in AIDS patients. It was licensed in Japan in 1993 but the licence was subsequently withdrawn after several deaths occurred in patients also receiving 5-fluorouracil as discussed earlier. The drug interaction was recognized before sorivudine was marketed, and the prescribing information clearly stated that sorivudine was contra-indicated in patients receiving 5-fluorouracil. It appears that medical practice in Japan involves a series of specialists dealing with different diseases in a single patient, so that the dermatologist treating shingles may be unaware that the patient is receiving anti-cancer therapy. To those of us in the West, this style of medicine appears incomprehensible and illustrates that extensive education of physicians about the risks as well as the benefits of antiviral chemotherapy is essential using information appropriate to each country.

**Cytomegalovirus**

**Transplant patients.** Since CMV infection is so common after transplantation and because CMV disease is so serious, although potentially treatable, surveillance cultures should be obtained from the time of transplantation onwards in order to detect infection at its earliest stage. A number of studies have shown that the detection of CMV in peripheral sites such as urine or saliva indicates a relative risk of about 2 for future CMV disease, whereas detection of CMV in the blood typically has a relative risk of about 7 for future disease (Meyers et al., 1990; Kidd et al., 1993; Pillay et al., 1993a).

There are thus four possible strategies for the use of drugs to control CMV disease (Table 4). Most controlled trials have reported prophylaxis to be effective so that

<table>
<thead>
<tr>
<th>Term used</th>
<th>When drug given</th>
<th>Risk CMV disease</th>
<th>Acceptable toxicity of drug</th>
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<tbody>
<tr>
<td>Prophylaxis</td>
<td>Before active infection</td>
<td>Low</td>
<td>Absent</td>
</tr>
<tr>
<td>Suppression</td>
<td>After peripheral detection</td>
<td>Medium</td>
<td>Low</td>
</tr>
<tr>
<td>Pre-emptive therapy</td>
<td>After systemic infection</td>
<td>High</td>
<td>Medium</td>
</tr>
<tr>
<td>Treatment</td>
<td>Once disease established</td>
<td>Established</td>
<td>High</td>
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