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Mini review

Herpes simplex virus latency and nucleoside analogues

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1. Introduction

Nucleoside analogues, such as acyclovir, famciclovir and valaciclovir are used clinically to treat or suppress herpes simplex virus (HSV) infections. We review here, the possibility that they might also be able to impact the establishment of latency and/or the timing of reactivations.

Experimental in-vivo studies have shown that acyclovir may reduce the establishment of latency when administered at a high dose and soon after virus inoculation, but this effect is lost if treatment is delayed (Klein et al., 1979; Field et al., 1979; Field and DeClerq, 1981). More recently, studies have been performed in HSV-infected mice to compare the efficacy of valaciclovir and famciclovir (oral pro-drugs of the active compounds acyclovir and penciclovir, respectively) (Field et al., 1995). In this model, famciclovir was more effective than valaciclovir in reducing the level of infectious virus at the peripheral site of inoculation (ear pinna) and within the brain stem. In addition, subsequent studies have shown that famciclovir is also more effective in reducing the establishment of latent HSV infection in mice and that treatment with famciclovir can be delayed for longer than valaciclovir and retain efficacy (Thackray and Field, 1996a).

The implications of these effects on the clinical situation are unknown. If the number of latently-infected neurons, or the number of HSV genome copies contained by the cells, can be decreased, it may have an effect on the rate and/or severity of

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recurrence. Clinical data concerning the effect of acyclovir treatment on recurrence are contradictory and there are, as yet, no clinical data on recurrence following treatment with famciclovir.

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2. Molecular biology of HSV latency

The ability of HSV to establish latency in the peripheral nervous system and periodically reactivate is critical to its survival in human populations. Many aspects of HSV pathogenesis and latency can be reproduced in animal models, including the mouse ear model described by Hill et al. (1975). In this model, the acute primary infection occurs in the ear pinna during which viral replication occurs. The virus then gains access to sensory nerves and travels via axons to establish infection in the sensory neurons which innervate the site of infection. Once in neurons, the virus can enter a productive cycle, resulting in the release of progeny virions, or establish true latency (Whitley, 1996).

Following the establishment of latency in humans, a number of stimuli can reactivate the virus resulting in entry into the lytic cycle, transport to the periphery, and multiplication, allowing the virus to disseminate to susceptible hosts. In the mouse model, latently-infected animals do not experience spontaneous recurrent disease at a high enough frequency for experimental purposes (Harbour et al., 1983; Openshaw, 1983; Shimeld et al., 1990). Reactivation of latent virus may be induced, however, by a number of methods, including nerve section, UV-irradiation, cellotape stripping (Blyth, et al., 1980), hyperthermic stress (Sawtell and Thompson, 1992) or by in vitro culture of explanted ganglia (Stevens and Cook, 1971; Field et al., 1979). The mouse model is, therefore, particularly useful for investigating events involved in the establishment and maintenance of latency at the cellular or molecular level and virus reactivation can be studied using a variety of inducible models.

2.1. Properties of latent viral DNA

Latent viral DNA, from the sensory ganglia of infected mice, is readily detectable by Southern blot hybridisation and appears stable in copy number over at least 4 months (Efstathiou et al., 1986). Using probes derived from the junction between the unique and repeat sequences of HSV DNA, terminal fragments cannot be found in latent DNA, demonstrating a structural difference between the DNA found in latent virus and in virion particles. The most likely explanation for this is that the viral DNA is circularised or exists as a concatamer during latency (Rock and Fraser, 1983, 1985; Efstathiou et al., 1986). The viral DNA is not integrated into the host cell genome (Mellerick and Fraser, 1987) but is organised in a structure similar in pattern to host nuclear chromatin (Deshmane and Fraser, 1989). Quantitatively, a DNA copy number of up to one viral genome per cell is detected by Southern blot hybridisation. However, since neuronal cells constitute only up to 10% of the cells in the ganglia and only a proportion of these will harbour latent DNA, each latently-infected neuron has been estimated to contain multiple copies of latent viral DNA (Roizman and Sears, 1987, 1996). Sawtell (1997) has recently used a corneal model of HSV infection in the mouse to analyse the frequency of infected neurons within one ganglion, and the viral DNA copy number within individual neurons. She found, using a contextual analysis in which latently-infected neurons were fixed and dissociated, that 1–30% of neurons harbour DNA (depending on the size of the original inoculum), and that each neuron contains <10–1000 genome copies/cell. More recently, this has been shown to be virus strain-dependent and to corre-

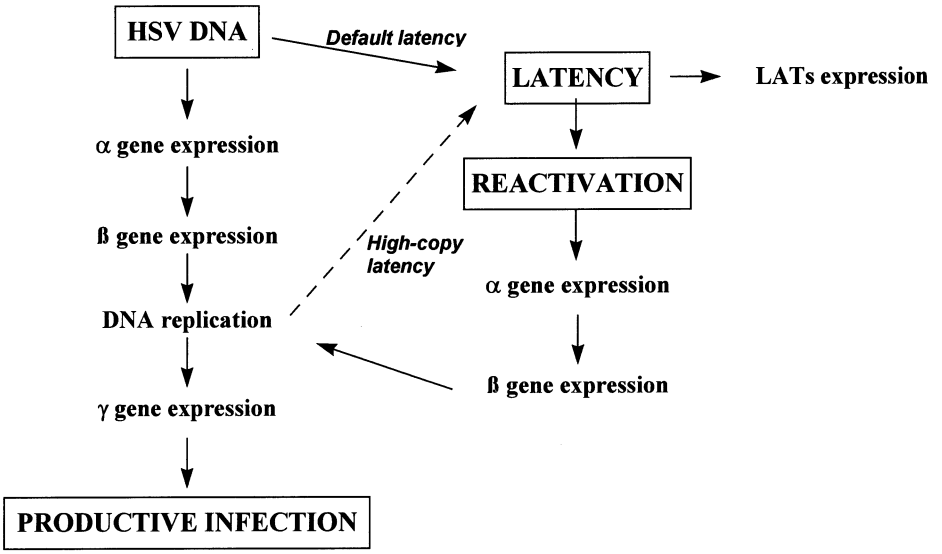

Fig. 1. Alternative routes for HSV DNA once it has entered the sensory neuron.

late with reactivation (Sawtell et al., 1998; Sawtell, 1998). An in-vivo model which makes use of the segmental sensory innervation of mouse flanks has been used to study the relationship between HSV DNA replication and the establishment of latent infection (Simmons et al., 1992; Slobedman et al., 1994). This model showed that there are a number of parameters which affect the levels of latent viral DNA in each neuron, including whether the site directly innervates the site of infection or is distal, the level of inoculum and expression of HSV thymidine kinase.

2.2. Default and high-copy latency

When the viral genome enters the sensory neuron, it can enter a productive, or lytic, cycle. This results in initiation of immediate early (IE) gene expression (α-gene products) which, in turn, initiate the production of β and γ-gene products. The outcome of this is DNA amplification, packaging of progeny genomes, and the release of infectious virus (Roizman and Sears, 1996) (Fig. 1). Studies using wild-type virus (Speck and Simmons, 1991, 1992; Simmons et al., 1992) and viral mutants with defects in IE gene expression (Katz et al., 1990; Ecob-Prince et al., 1993) have shown that viral gene expression is not a prerequisite for the establishment of latency. Thus, failure of virus IE gene expression can result in establishment of latency-the so called 'default mechanism' of latency establishment (Fig. 1). It is relatively simple to model the default mechanism, and a number of ways in which a default pathway could be entered can be hypothesised. Functional Vmw65, cellular Oct-1 and CFF proteins are required to trigger IE gene expression, so the loss of Vmw65 during retrograde axonal transport or the lack of Oct-1/CFF proteins in the nuclei of post-mitotic sensory neurons could result in default latency. Sears et al. (1991) showed that constitutive expression of Vmw65 does not prevent the establishment of latency, however, there may be other negative factors which combine to prevent the formation of the complexes necessary for gene expression (reviewed by Steiner and Kennedy, 1995). For example, other sensory neuron-specific Oct-binding proteins (e.g. Brn3 or Oct-2) may act as direct negative factors or compete with and inhibit Oct-1/Vmw65 complexes from forming (Latchman, 1994).

As well as the default mechanism, latency may be established after limited gene expression within the neuron, resulting in high-copy number latency

(Simmons et al., 1992; Slobedman et al., 1994). Once latency has been established, there is no evidence of lytic viral gene expression and only one area of the genome has been found to have appreciable transcriptional activity during latency, and this encodes the latency-associated transcripts (LATs) (Deatly et al., 1987; Stevens et al., 1987).

2.3. Latency-associated transcripts

HSV LATs have been extensively reviewed by Fraser et al., 1992 and more recently by Block and Hill, 1997. The region of the genome encoding the LATs lies in the repeats which flank the unique long region, making the LATs diploid in copy number. HSV-encoded LATs consist of a series of nuclear transcripts comprising two highly-abundant, non-polyadenylated species of 2.0 and 1.5 kb, termed major LATs, which are transcribed antisense to part of the ICPO immediate early gene. The precise mechanism of synthesis of major LATs is unclear, although there is evidence to support the view that these transcripts represent stable introns derived from a less abundant 8.5 kb polyadenylated precursor RNA termed minor LAT (Fraser et al., 1992). There is no evidence of viral protein expression in latently-infected neurons in-vivo and the function of these transcripts in viral pathogenesis remains uncertain. LAT-negative mutants have been found to replicate efficiently in cell culture and to establish, and reactivate from, latency (Leib et al., 1989; Steiner et al., 1989; Krause et al., 1995). There are considerable data, however, which support the view that the LAT region is required for the efficient spontaneous or induced reactivation of latent virus, as assessed in a number of animal model systems (Leib et al., 1989; Perng et al., 1994; Krause et al., 1995; Hill et al., 1996). Nevertheless, more recent, quantitative studies examining latency at the neuronal level, suggest that the role of LATs is to increase the number of neurons in which latency is established. Thus LAT-negative mutants may reactivate less efficiently than wild type virus as a direct consequence of latency being established in fewer neurons (Thompson and Sawtell, 1997; Sawtell, 1998).

2.4. Conclusions

When the virus enters a neuron, default latency, high-copy latency or productive infection can result. The former is unlikely to be affected by therapy with nucleoside analogues, whereas high-copy latency, which may depend on a limited burst of virus gene expression before latency is established, and productive infection could be affected. In all cases, if the viral load is decreased by the action of a nucleoside analogue during the replication stage, both at the peripheral infection site and in the ganglia, the amount of virus available to establish latency could be decreased.

3. Experimental and clinical data with acyclovir

Many studies have shown the benefits of acyclovir treatment on the acute symptoms of HSV infection in man. A number of experimental and clinical studies have been conducted to investigate whether acyclovir treatment can affect the establishment of latency and/or prevent or reduce recurrences. These are summarised in Table 1 and are described below.

3.1. Experimental data

3.1.1. Latency

Field et al. (1979) used the mouse ear pinna model to study the effects of acyclovir 50 mg/kg, administered subcutaneously or intra-peritoneally for 11 days starting 1 day before inoculation of HSV-1. Once latency was established, cervical ganglia were removed, held ex-vivo for 5–6 days, homogenised and virus titres determined. Subcutaneous administration of acyclovir reduced virus titre in the ganglia (2.1 $\log_{10}$ in treated versus 3.6 $\log_{10}$ in untreated mice), whereas intra-peritoneal administration reduced both virus titre and the proportion of latently-infected mice to the limit of detection. Thus, acyclovir had an effect on the establishment of latency in-vivo when dosing was started prior to viral exposure. It was also noted in this paper that the ability to prevent the establishment of latency was dependent on the dose of virus used to inoculate the mice.

Table 1
Experimental and clinical data on the efficacy of acyclovir treatment against HSV latency and recurrence

Model /indication (reference)	Acyclovir dosing details				Effects observed[b]
	Time of initiation[a]	Dose level	Route	Duration [days] (freq.)	
Experimental models					
Mouse ear pinna (Field et al., 1979)	−24 h	50 mg/kg per day	s.c.	11	Slight effect on virus titre
	−24 h	50 mg/kg per day	i.p.	11	Marked reduction of% mice with latent virus and virus titre
Mouse lip model (Park et al., 1979)	+24 h	20 mg/kg per day	s.c.	4 (bid)	No effect
	+24 h	40 mg/kg per day	s.c.	4 (bid)	Slight effect on latency
	+24 h	60 mg/kg per day	s.c.	4 (bid)	Significant reduction in% mice with latent infection
Mouse cornea model (Pavan-Langston, et al., 1981)	+3 h	60 mg/kg per day	s.c.	5	Significant reduction in latency
	+24 h	60 mg/kg per day	s.c.	5	Slight decrease in latency cf control
Guinea-pig vaginal model (Bourne and Stanberry, unpublished)	+12 h	60 mg/kg per day	i.p.	7 (bid)	Significant reduction in primary disease and latency cf control
Guinea-pig vaginal model (Bourne and Stanberry, unpublished)	+24 h	80 mg/kg per day	i.p.	10 (bid)	Significant effect on primary disease but no effect on recurrence
Clinical studies					
Primary HSV-2 genital herpes (Corey et al., 1983a) ($n = 31$)	$\leq$7 days	5 mg/kg	i.v.	5 (tid)	Some effect on recurrence, particularly in patients treated $\leq$7 days after sexual exposure
Primary HSV-2 genital herpes (Corey and Mindell, 1985) ($n = 40$)	$\leq$7 days	5 mg/kg	i.v.	5 (tid)	Trend towards reduction in recurrences seen in previous study was lost
Primary HSV-2 genital herpes (Peacock et al., 1988) ($n = 45$)	$\leq$7 days	5 mg/kg	i.v.	5 (tid)	No effect seen on recurrence time or incidence
Primary HSV-2 genital herpes (Corey et al., 1983b) ($n = 60$)	$\leq$7 days	200 mg	p.o.	10 (5 × day)	No significant effects on time to recurrence or rate of recurrence
Primary HSV-2 genital herpes (Bryson et al., 1985) ($n = 21$)	$\leq$6 days	200 mg	po	10 (5 × day)	No significant effect on recurrence up to 6 months but significant effects on recurrence rates between 6 and 24 months after the start of treatment
Primary HSV-2 genital herpes (Kinghorn et al., 1986) ($n = 18$)	$\leq$6 days	200 mg	p.o.	7 (5 × day)	No effect on recurrence
Primary genital herpes (Wald et al., 1992) ($n =$ 87)	$\leq$5 days	800 mg, then 200 mg	p.o.	10 (5 × day)	No significant effect on time to first recurrence cf 800 mg (5 × day), then placebo (5 × day)

[a] Time post-inoculation (experimental models) or post-lesion appearance (clinical studies).
[b] 'Significant' indicates $P < 0.05$.

In the same year, Park et al. (1979) used a mouse lip model of HSV-1 infection and delayed the start of acyclovir dosing until 24 h post-inoculation. They showed that subcutaneous administration of 60 mg/kg acyclovir, given twice daily for 4 days, had a significant effect ($P < 0.05$) on the establishment of HSV-1 latency (36% in treated mice and 79% in untreated mice) but that lower doses of 20 and 40 mg/kg were not significantly effective. This study demonstrated that acyclovir could have an effect on the establishment of latency, even when the drug was administered 24 h post-exposure.

Using another mouse model (HSV-1 infection of the cornea), Pavan-Langstan et al., 1981 investigated the effect of timing of the first dose on efficacy. Acyclovir was administered subcutaneously at 60 mg/kg, starting at either 3 or 24 h post-inoculation and continuing for 5 days. After 21 days, the trigeminal ganglia were explanted and latent virus detected by cocultivation. All untreated mice and 58% of mice treated from 24 h post-inoculation (not significantly different to the controls) were latently infected. In contrast, only 21% of mice treated from 3 h post-inoculation were latently infected ($P < 0.05$). Thus, early initiation of treatment appeared to have a greater effect on the establishment of latency.

Bourne and Stanberry used a guinea pig vaginal challenge model of HSV-2 infection (unpublished data) to assess the effect of acyclovir on primary and latent infection. In the treated group, guinea pigs were administered 60 mg/kg intra-peritoneal acyclovir twice daily for 7 days, starting at 12 h post-inoculation with virus expressing β-galactosidase, before any symptoms of disease were apparent. The severity of the primary infection was determined by the area under the curve (AUC) of the lesion score-day curve, and the latency by enumeration of latently-infected sacral dorsal root ganglia neurons expressing b-galactosidase 30 days post-inoculation. The severity of the primary disease was significantly reduced following treatment with acyclovir ($P < 0.05$). In addition, the number of latently-infected neurons in the acyclovir-treated group (34.1) was significantly reduced compared with the untreated control group (154.9).

3.1.2. Recurrence

Recurrence of infection was examined in a study in the guinea pig model of genital herpes in which acyclovir was dosed twice daily at 80 mg/kg per day intra-peritoneally for 10 days starting 24 h post-inoculation (Bourne and Stanberry, unpublished data). The severity of the primary disease was again assessed as the AUC of the lesion score-day curve and, after recovery from acute infection, the animals were followed for a further 40 days to determine the frequency of spontaneous recurrent infection. The severity of the primary disease was significantly less in the acyclovir-treated group, but the mean number of days on which recurrent infections occurred was not significantly different for guinea pigs treated with acyclovir (9.7 days) compared with untreated guinea pigs (9.0 days). Acyclovir administered at a relatively high dose during primary infection therefore had no effect on recurrence of infection in this model. This confirmed an earlier study (Bernstein et al., 1986), in which acyclovir was given, in the animals' drinking water, early after inoculation and had no effect on HSV recurrence assessed after acyclovir treatment was stopped.

3.1.3. Conclusions

These animal model studies demonstrate that acyclovir treatment of the acute infection can affect the establishment of latency but that this effect is dependent on the timing of initiation of treatment, the amount of drug administered, the route of administration, the duration of treatment and the amount of virus inoculated. Acyclovir treatment, when initiated soon after infection, appears to reduce latent infection rather than prevent it, and an effect on future recurrent infection has not been demonstrated in any of the animal models.

3.2. Clinical data

Data from animal models cannot always be directly applied to humans. For example, in the clinical situation, treatment cannot normally be initiated before exposure to virus. Animal studies provide more precise, quantitative data, however, since direct measurement of latent virus in the

ganglia can be performed and effects on known primary infection and known recurrence can be compared. In humans, the first symptomatic episode of genital herpes may not always be a primary infection, since the primary infection may have been asymptomatic. It is important to determine whether this is the case when studying the infection in humans. In addition, since latent virus cannot be quantified directly in clinical studies, time to recurrence and the number of recurrent episodes have been used to indirectly assess latent virus.

3.2.1. Intravenous acyclovir

Corey et al. (1983a) looked at the rate of recurrence in 31 patients with primary HSV-2 genital herpes who had lesions for < 7 days before treatment. Intravenous acyclovir was administered at 5 mg/kg, 3 times/day for 5 days. During the follow-up period (10–11 months), 60% of patients treated with acyclovir and 88% of patients treated with placebo experienced a recurrence and the rate of recurrence per month was 0.26 in the acyclovir-treated group compared with 0.5 in the placebo-treated group. Moreover, four of seven patients who had received acyclovir within 7 days of sexual exposure to virus experienced no recurrences during the follow-up period, suggesting that time from exposure to the start of treatment, rather than time from the appearance of lesions to the start of treatment, may be an important parameter. Although none of the differences were statistically significant, there was a trend and it was felt that a larger number of patients should be analysed. These data were therefore combined with results from a study conducted in 30 patients in the UK by Mindel et al. (1982), who used the same entry criteria and dosing schedule, but the trend towards an advantage for treatment with acyclovir was lost when the two studies were combined (Corey et al., 1985).

A multicenter study was reported by Peacock et al. (1988), in which 45 patients with primary genital HSV-2 infection who had had lesions for < 7 days were given either 5 mg/kg acyclovir or placebo intravenously 3 times/day for 5 days. No difference was seen between the placebo and acyclovir-treated groups for the proportion of patients with a recurrence, the time to recurrence or, the mean monthly incidence of recurrence. Thus, these clinical studies did not produce clear evidence that intravenous acyclovir treatment of the first episode reduces recurrences, although there was a suggestion of some effect if therapy was started early enough.

3.2.2. Oral acyclovir

A clinical study of 60 patients with primary HSV-2 genital infection, whose lesions had been present for < 7 days, examined recurrences of genital herpes during the 4 months following treatment of the first episode (Corey et al., 1983b). Patients received either placebo or 200 mg acyclovir orally 5 times per day for 10 days. No significant differences were seen between acyclovir treatment (0.31 recurrences/month) and placebo (0.27 recurrences/month). Subsequently, Bryson et al. (1985) found more encouraging results, using the same dose and regimen of acyclovir to treat 21 patients with primary HSV-2 genital infection whose lesions had been present for < 6 days. They looked at the number of recurrences which occurred up to 2 years after treatment and, as in the previous study (Corey et al., 1983b), found no significant difference between the acyclovir- and placebo-treated groups for recurrences up to 6 months. The rate of recurrence of infection in the two groups was significantly different, however, between 6 and 12 months ($P < 0.01$), 12 and 18 months ($P < 0.001$) and 18 and 24 months ($P < 0.05$) after treatment. Overall, the proportion of patients experiencing recurrences up to 1 year was 70% in the placebo group and 12.5% in the acyclovir-treated group, suggesting that treatment of the first episode with acyclovir could have an effect on the frequency of subsequent recurrences. These results should be interpreted with caution, however, bearing in mind the small number of patients studied.

Kinghorn et al. (1986) examined the effect of 200 mg acyclovir administered orally 5 times per day for 7 days on the rate of recurrence in 18 patients with primary HSV-2 infection whose lesions had been present for < 6 days at the time of treatment. In contrast to the data reported by Bryson et al. (1985)no significant difference com-

pared with placebo was seen. The recurrence rate during the 12 month follow-up was higher (100%; 7/7) in the acyclovir-treated group than in the placebo-treated group (73%; 8/11).

Thus, the clinical data for the standard dose of acyclovir obtained in three studies showed conflicting results and raised two questions: what would be the effect of increasing the dose of acyclovir and, was dosing started early enough? In the clinical setting, it is difficult to start treatment of patients with first episode genital herpes earlier, but the question of dose level could be studied. Wald et al. (1994) treated 87 patients with primary genital herpes whose lesions had been present for ≤ 5 days. Two thirds of the patients received 800 mg acyclovir five times a day for 10 days and were then randomised to 200 mg or placebo thereafter. The remaining third received 200 mg acyclovir per dose throughout. Disappointingly, there were no significant differences in the time to first recurrence between the three groups.

3.3. Conclusions

Studies in animal models show that acyclovir treatment initiated before or shortly after exposure to the virus can reduce the magnitude of the infection and the number of latently-infected neurons. This effect appears to be highly dependent on a number of factors, however, including the level and route of dose administered and timing of dosing relative to inoculation. Clinical data show that treatment of the first episode with acyclovir has, at best, only a modest effect on recurrence rates. The factors likely to be important in affecting latency are the interval between sexual exposure to virus and initiation of drug treatment, the dose and potency of the antiviral drug, its delivery to neural tissues and effect in blocking the establishment of latent infection.

4. Experimental data with famciclovir and valaciclovir in mice

Famciclovir and valaciclovir, prodrugs of penciclovir and acyclovir respectively, have recently become available for treatment of herpes infections. In plaque-reduction assays in 3T3 cells, which are derived from BALB/c mice, the potencies of penciclovir and acyclovir are similar, with EC_{50} values of 0.02 and 0.01 mg/ml, respectively, against HSV-1. Famciclovir and valaciclovir also give similar concentration-time curves (AUC), and elimination rates for the active components in blood, following oral administration of 50 mg/kg to mice, with concentrations falling below the limit of detection by 8 h (Field et al., 1995). Comparative effects of these compounds on the establishment of latency have been examined in the murine model of HSV infection via the ear pinna.

4.1. Efficacy against primary HSV-1 infection and subsequent latency in mice

In a study of 680 BALB/c mice, famciclovir or valaciclovir were administered via the drinking water to give between 100 and 150 mg/kg per day for each compound. The compounds were administered from Days 1, 2, 3, 4 or 5 post-infection up to Day 10, and the progress of the infection was followed in the acute phase. Three to 4 months later, the ganglia were removed and tested for latency by means of explant culture (Thackray and Field, 1996a).

Compared to untreated controls, both nucleoside analogues affected clinical features. When comparing the two drugs, however, valaciclovir was less effective than famciclovir regarding neurological signs, weight gain and, in particular, mortality. For mice treated with valaciclovir, the survival rate was dependent on the timing of the start of treatment, being higher in mice treated from Day 1 than in mice treated from Day 5. In contrast, all famciclovir-treated mice survived irrespective of the time of initiation of treatment. During the acute infection, virus growth in the ear pinna was similar for both agents but, in the brain stem, infectious virus was completely suppressed when famciclovir treatment was initiated at < 3 days whereas this was not the case following valaciclovir treatment (Fig. 2). In addition, a transient burst of virus replication was seen in the brain stem on cessation of valaciclovir treatment,

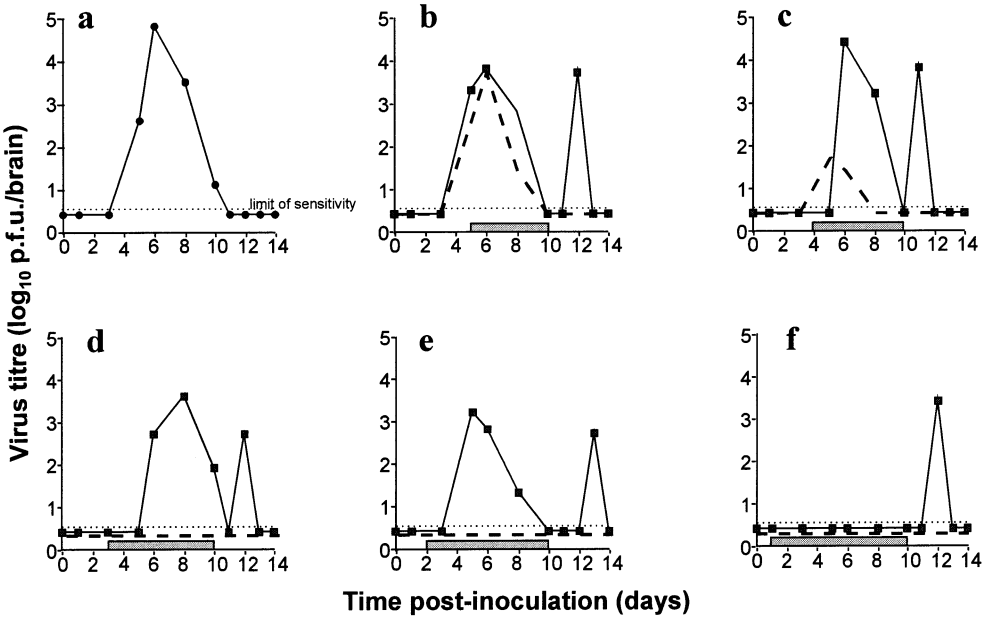

Fig. 2. Effect of famciclovir (----) or valaciclovir (■) therapy on the titre of HSV-1 in the brain stem of mice infected via the ear pinna, compared with untreated mice (●) (a). Therapy was started at 5 (b), 4 (c), 3 (d), 2 (e) or 1 (f) days post-inoculation and continued up to 10 days post-inoculation (▨). Adapted from Thackray and Field, 1996a

irrespective of the time at which dosing was started (Fig. 2). This transient rebound of infectious virus was not seen in mice treated with famciclovir.

The ganglia of the mice were explanted 3–4 months after the initiation of therapy, incubated for 4–5 days and tested for the presence of infectious virus. No virus was reactivated from any of the mice treated with famciclovir from < 3 days and there was a significant reduction in the proportion of mice from which latent virus could be reactivated when famciclovir treatment was delayed for 5 days (Table 2). This was true for both trigeminal and dorsal root ganglia and no latent virus was detected in the contra-lateral ganglia for any of the famciclovir-treated mice. In contrast, 38–75% of mice treated with valaciclovir from Days 1 or 2 contained virus in the ganglia which was reactivated on cocultivation. Later treatment with valaciclovir (from 4 or 5 days post-inoculation) was ineffective (Table 2). These data are supported by results from other workers (Sutton et al., 1998) who, using transient hyperthermia, showed that HSV-1 could be reactivated from the ganglia of significantly ($P < 0.05$) fewer mice treated with famciclovir from 2 to 10 days post-inoculation (4/17; 24%), compared with valaciclovir-treated animals (12/19; 63%) or placebo (13/18; 72%).

4.2. Efficacy against HSV-2

Data have also been obtained in the ear pinna model for HSV-2 infection (Thackray and Field, 1996b). Latent virus could not be reactivated from the lateral and contra-lateral, dorsal and trigeminal, ganglia of mice treated with famciclovir (50 mg/kg bid for 5 days, from 22 h post-inoculation), whereas latent virus was reactivated from at least one ganglion in 60% of mice treated with the same dose of valaciclovir.

4.3. Sensitive methods for the detection of latent viral DNA

Thackray and Field, (1998) have used four different methods to determine the amount of latent virus in the ganglia of HSV-1 infected mice.

Table 2
Proportion of HSV-1-inoculated mice from which reactivated latent virus was cultured 3 to 4 months after primary infection of the left ear and treatment with valaciclovir (100–150 mg/kg per day) or famciclovir (100–150 mg/kg per day)[a]

Treatment	Trigeminal ganglia ($n = 16$)		Dorsal root ganglia ($n = 16$)	
Days post-inoculation	Left	Right	Left	Right
None	100[b]	36	100	44
Valaciclovir				
5–10	100	25	100	100
4–10	100	75	100	75
3–10	88	50	75	56
2–10	38	0	44	0
1–10	69	0	75	0
Famciclovir				
5–10	38	0	38	0
4–10	13	0	13	0
3–10	0	0	0	0
2–10	0	0	0	0
1–10	100[b]	0	0	0

[a] Adapted from Thackray and Field, 1996a.
[b] % of mice.

They have used the explantation/cocultivation method described above and the following: long-term culture (14 weeks); enzymatic ganglia disaggregation and measurement of infectious centres; and, in-situ hybridization for LAT-positive neurons. In this experiment, valaciclovir or famciclovir was given at 50 mg/kg by oral gavage b.i.d. The infectious centre method detected 95% fewer HSV-infected cells in ganglia from mice that had been treated with famciclovir from two days after infection compared with untreated controls. It was of interest, however, that no virus was reactivated from the same group of famciclovir-treated mice using the method of explant culture for 4–6 days. In fact, all ganglia were positive for latency using the most sensitive methods although at different levels depending on the treatment administered (Field et al., 1998). These results confirmed that latency is likely to be reduced, rather than eliminated, by treatment with nucleoside analogues.

4.4. Conclusions

For both famciclovir and valaciclovir, early treatment reduced the amount of latent virus in the ganglia compared with placebo, although the effects were more marked with famciclovir by all four methods of detection. Famciclovir reduced the number of latently-infected neurons when initiation of treatment was delayed for 5 days post-inoculation, whereas valaciclovir treatment had no effect when treatment was initiated later than 3 days post-inoculation. The reason(s) for the difference between famciclovir and valaciclovir is uncertain. There may be pharmacological differences between the two drugs, such as a dose effect due to differences in drug metabolism or differences in the distribution of acyclovir and penciclovir to neural compartments, but there are no data currently available to support these possibilities. There are biochemical differences between the two drugs, however, such as enzyme affinities and intracellular half-life of the triphosphates (Table 3). Alternatively, there could be a molecular explanation regarding how the drugs interact with the virus-infected neurons.

It is difficult to extrapolate murine data to man without knowing the mechanism causing the differences in efficacy between famciclovir and valaciclovir in mice. There may be a window of opportunity, however, for early therapy with nu-

Table 3
Comparative biochemical properties of acyclovir and penciclovir

Parameter	Acyclovir	Penciclovir
Concentration in neural tissue	?	?
Intracellular triphosphate half life	Short (0.7 h)	Long (10 h)
Affinity of nucleoside for thymidine kinase (K_m)	Low (172 μM)	High (1.5 μM)
Affinity of the triphosphate for inhibiting DNA polymerase (K_i)	Very high (0.07 μM)	low (8.5 μM)
Obligate chain terminator	Yes	No
Fate of infected cells	?	?

cleoside analogues to affect the establishment of latent infection and the subsequent pattern of recurrent disease. Whether or not the differences seen between the two drugs can be translated to the clinical setting will only become apparent when tested in a clinical trial.

5. Discussion

Latency of HSV can therefore be established via two alternative mechanisms. Default latency, which does not require expression of viral genes, and high-copy latency, which occurs after some viral replication has occurred in the neurons. It is thought that nucleoside analogues will not affect default latency but that they may affect high-copy latency. It is not known, however, which form of latent virus is important in recurrent disease and whether inhibition of high-copy latency would have a clinically significant effect on the rate or severity of HSV recurrence.

5.1. Relationship between latency and recurrence

5.1.1. Quantitative studies

A number of studies have been performed to look at these questions, including those described by Sawtell, 1997 who examined the effect of acyclovir on the establishment of latent infection using single-cell polymerase chain reaction. An HSV-1 corneal infection in mice was treated with 50 mg/kg acyclovir three times daily, administered intra-peritoneally from various times after inoculation. The reduction in the number of latently-infected neurons in the trigeminal ganglia was greatest when treatment was started at the time of inoculation (Table 4) and was progressively less marked as the initiation of treatment was delayed further. No reduction in the number of latently-infected neurons found in the trigeminal ganglia was seen when acyclovir treatment was delayed for ≥ 4 days post-inoculation, which is consistent with the results reported by Klein et al. (1979) and those described by Thackray and Field (1996a)(Fig. 2). In addition, when treatment was started at the time of inoculation, a significant decrease in the number of viral genome copies per

Table 4
Reduction in the number of neurons infected with latent HSV-1, and viral genome copies per neuronal cell, following treatment of mice with acyclovir starting one to six days post-inoculation of the cornea[a]

Time of first acyclovir dose (post-inoculation)	Reduction in number of latently-infected neurons	Viral genome copies per latently-infected neuronal cell
0	20-fold	<10
1	15-fold	<10
1.5	10-fold	<10–100?
2	4-fold	<10–100?
3	3-fold	<10–100?
4	None	10–1000
5	None	10–1000

[a] Adapted from Sawtell, 1997.

latently-infected neuronal cell was seen (Table 4). It is possible that this reduction in copy number represents the loss of high-copy latency, leaving only latency established via the default mechanism.

The rate of hyperthermic stress-induced recurrence of HSV infection was also studied in this model to determine the relationship between latency burden and reactivation. Animals treated with acyclovir from 0 to 36 h post-inoculation showed significantly less recurrence of infection, suggesting that the number of latently-infected neurons and/or the viral genome copy number within the neurons were linked to the rate of recurrence. When plotted, a good correlation ($r = 0.9852$; $P < 0.0001$) was seen between the number of animals in which reactivation was seen and the frequency of latently-infected neurons (Sawtell, 1998). The frequency of in vivo reactivation was also found to be affected by the viral input titre, such that the higher the inoculum level, the greater the number of latently-infected neurons and the greater the recurrence rate. For example, 70% of mice reactivated when the input inoculum was 4×10^5 pfu/ml, whereas only 30% of mice showed reactivation of latent virus when the inoculum was 2×10^3 pfu/ml. Interestingly, when a single dose of acyclovir was given between 6 and 9 h post-temperature shock in the same model, the production of detectable infectious virus in the ganglia (reactivation) was eliminated in approximately 80% of mice. Little effect was seen when the dose of acyclovir was given at either 3 or ≥ 18 h post-temperature shock, demonstrating the importance of the timing of drug administration.

5.1.2. Thymidine kinase-deficient variants of HSV

Another way to examine the relationship between latency and recurrence is to look at variants of HSV which are thymidine kinase-negative (Efstathiou et al., 1989; Tenser, 1991). Thymidine kinase is a non-specific neuro-virulance determinant, required for viral replication inside neuronal cells. In theory, a thymidine kinase-negative strain of HSV will show normal replication at the peripheral site, but restricted replication in the neuron. Thymidine kinase deficiency should not, therefore, influence default latency but high-copy latency may be affected, since it relies on viral replication inside the neuron. Reactivation could also be affected since, although initiation of immediate early gene expression may occur, there would be reduced viral replication and, therefore, no symptoms. Problems which have confounded studies with thymidine kinase-negative variants are: the lack of studies using well-characterised strains; the finding that thymidine kinase-negative variants can produce thymidine kinase-positive variants following passage in-vivo; in-vivo complementation, which 'helps' thymidine kinase-negative mutants used in animal models; and, the possibility that viral DNA could be amplified through Ori H (Sears and Roizman, 1990). Nevertheless, compounds which inhibit thymidine kinase, although they do not inactivate the virus, have been shown to decrease the rate of reactivation (Martin et al., 1998; Watkins et al., 1998) as well as the rate of recurrence in animal models (Stanberry, unpublished data).

5.2. Relevance of experimental in-vivo data

Most of the detailed data described have been produced in animal models, since this is the only way to assess effects on numbers of latently-infected neurons and viral genome copy number within these cells. It is debatable, however, whether these data are representative of the situation in man. In an animal model, the time of inoculation is known and treatment can be administered at a precise time before or after virus inoculation. The clinical situation is very different. In man, lesions do not appear until approximately 5 days after sexual exposure to the virus and this is generally the first indication that an infection is present. It is not known in humans, however, whether the appearance of lesions is pre-or post-latency, i.e. has the virus only replicated at the peripheral site or has it travelled to the ganglia, replicated there, produced latent infection, and then returned via the axons to the peripheral site. Acyclovir treatment has been shown, in animal models, to affect not only viral replication at the peripheral site but also viral replication in the ganglia, but treatment as soon as possible after sexual exposure to the virus is of key importance.

5.3. Effect of nucleoside analogues

Treatment with nucleoside analogues reduces viral replication at both the peripheral and ganglionic sites. The overall titre of virus available for either default or high-copy latency could therefore be reduced and the viral replication required to give high-copy latency could also be inhibited. Not only is it important, however, to take advantage of the 'window of opportunity' and initiate dosing at the earliest opportunity, but it is also important to maintain inhibitory concentrations of the nucleoside analogues at the site of replication for as long as possible. Any rebound of virus replication and resultant latent infection, which occurs in the concentration troughs, may offset the advantage of early initiation of treatment. The sites of viral replication are inside the epithelial and neuronal cells. Therefore, the 10-fold longer intracellular half-life of penciclovir triphosphate (the active component of famciclovir) compared with acyclovir triphosphate (the active component of valaciclovir) may explain the greater effect on the establishment of latency of famciclovir compared with valaciclovir seen in the mouse ear pinna model. Moreover, in this in-vivo model, famciclovir was effective at reducing latency even when treatment was delayed for up to 5 days post-inoculation whereas valaciclovir was only effective when dosed < 2 days post-inoculation. This suggests that the window of opportunity may be longer for famciclovir than for valaciclovir.

Although continuous exposure to drug throughout the first episode of acute HSV infection may be necessary, indefinite treatment may not. It has been shown that when patients come off long term therapy with acyclovir, they may experience lower recurrence rates than before treatment (Fife et al., 1994). Also, animal models of HSV pathogenesis have shown loss of latent infection in the ganglia over time using quantitative PCR (Stanberry, unpublished data). This may be because there is destruction of latently-infected neurons with each reactivation event, and these events do not recharge the numbers of latently-infected neurons, or it may be because old neurons do not reactivate as well as new neurons. Published data (Hill et al., 1996) indicate that the reservoir of latent viral DNA is stable, but further studies are required to confirm this, as well as to determine the state (static or dynamic equilibrium) of the latent viral DNA reservoir.

Overall, a number of experimental in-vivo models have shown that acyclovir may have an effect on the establishment of latent HSV, but clinical studies have not confirmed an effect on recurrence of infection following treatment of the first episode. Recent animal studies have shown an advantage for famciclovir over valaciclovir, however, in the reduction of latent infection. The clinical efficacy of famciclovir and valaciclovir in man is therefore currently being assessed in patients with first episode genital herpes, presenting within 72 h of lesion onset and within 2 weeks of sexual exposure. Only patients with primary HSV-2 infection are included in the analysis of the 10-month, post-healing follow-up phase. No placebo control could be included in this study for ethical reasons so, if the study does not show a difference between the two treatment groups, interpretation of the results will be difficult. It will not be clear whether there is no difference between the two drugs, or whether the primary disease treated is post-latency and nucleoside analogue treatment is therefore ineffective. It is possible, however, that early treatment of HSV genital infection with famciclovir could have an effect on recurrence rates in man, mirroring the results seen in mice. The results of the clinical study are expected in 1999 and are awaited with interest.

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