A Quantitative Study of the Effects of Several Nucleoside Analogues on Established Herpes Encephalitis in Mice

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(Accepted 24 January 1984)

SUMMARY

Mice with established herpes encephalitis were used to compare the effects of chemotherapy using three different nucleoside analogues. Encephalitis was produced by intranasal inoculation of a type 1 strain of herpes simplex virus. Without chemotherapy all mice died within 5 to 7 days of inoculation. Oral acyclovir (ACV) was a successful preventative measure if commenced within 2 days of inoculation but much less effective if the onset of treatment was further delayed. From the third day, when central nervous system infection had definitely become established, ACV only reduced mortality if given intraperitoneally (i.p.) at regular 6-hourly intervals. Comparison with bromovinyldeoxyuridine (BVdU) and the new nucleoside analogue dihydroxypropoxymethylguanine (DHPG) using the same 6-hourly i.p. regimen revealed that BVdU was poorly effective, despite better activity in vitro, whereas DHPG was the most successful. Virus was rapidly eradicated from all parts of the brain by DHPG therapy, and by day 10, no infectious virus remained in the brains of treated mice, no virus antigens were observed and no trace of virus DNA could be detected in neural tissues by Southern blotting.

INTRODUCTION

Human herpes encephalitis is a life-threatening disease and as much information as possible must be sought from animal models in order to suggest the most effective forms of therapy for trial in man. Until recently the only useful compound available for systemic therapy was adenine arabinoside (AraA); some success was obtained with this (Whitley et al., 1977, 1981), but the compound has the disadvantage of being poorly soluble and is susceptible to deaminase attack in vivo which converts the nucleoside to a much less active form (Glazko et al., 1975). Therefore there is much interest in several new and highly potent agents which are active against herpes simplex virus (HSV). Acyclovir (ACV) was shown to be effective against herpes encephalitis in mice (Schaeffer et al., 1978; Park et al., 1979; Kern et al., 1982) and trials in man are already underway (Whitley, 1983). However, two other compounds have emerged whose apparent lack of toxicity may also allow systemic use. Bromovinyldeoxyuridine (BVdU) (de Clercq et al., 1982); Reefschlager et al., 1982) and dihydroxypropoxymethylguanine (DHPG or 2'NDG) (Ashton et al., 1982) have both been reported to prevent herpes encephalitis in mice. However, the majority of published work to date has principally involved only the survival of infected mice as a measure of the effectiveness of chemotherapy and little quantitative data has been reported on the degree of inhibition of virus replication within the central nervous system (CNS) or the extent of tissue damage in the surviving animals.

An important aspect of human viral encephalitis is the difficulty of specific diagnosis especially in the early stages of the disease (Whitley et al., 1981) and thus a realistic model for the treatment of human disease should involve a period of active virus replication in the CNS prior to commencing therapy. The present paper describes such a model in which a potentially fatal encephalitis is established in mice following intranasal inoculation of type 1 HSV (de Clercq & Luczak, 1975). The model is well characterized in terms of the distribution of virus antigen and infectious virus at various times after initiating the infection, with a pattern of infection that
closely parallels human encephalitis (Esiri, 1982). These mice were used to demonstrate that effective chemotherapy is possible after extensive virus replication is established in the CNS and it also enabled the quantitative comparison of three different nucleoside analogues (ACV, BVdU and DHPG) which were used to treat the infection.

METHODS

Virus and mouse inoculation. The virus was HSV-1 SC16 (Hill et al., 1975). Virus infectivity was measured by plaque titration in BHK-21 cells; for mouse inoculation virus was diluted in Eagle's minimal essential medium (MEM) without serum. Intranasal inoculation was carried out by instilling 20 ~l of virus suspension into each nare of lightly anaesthetized mice. The BALB/c female mice aged 3 to 4 weeks were obtained from Bantin & Kingman, Grimston, Aldbrough, Hull, U, K. The inoculum contained 109 p.f.u. which corresponds to approx. ten times the LD50 dose by this route (Anderson & Field, 1983).

Measurement of virus titre in tissues. Tissue samples containing virus were stored dry at -70 °C. Samples were later ground in 1 ml MEM in small conical glass grinders and the presence of infectious virus was determined by adding dilutions of the tissue homogenates to BHK-21 cells. The cells were allowed to form monolayers in 5 cm plastic Petri dishes and after 2 days incubation at 37 °C the cultures were fixed, stained and plaques counted.

Detection of latent HSV in neural tissue. The trigeminal and dorsal root ganglia were explanted directly into small bottles containing 0.5 ml MEM with 1% calf serum. The eyes were also maintained whole but larger tissues (olfactory lobes and brain stems) were divided into several pieces. The cultures were incubated at 37 °C in equilibrium with humidified 5% CO2 for 6 days then homogenized and tested for the presence of infectious virus as described above. These methods were found previously to be extremely effective for the detection of latent HSV in cervical or lumbar-sacral dorsal root ganglia after skin inoculation (Field & Wildy, 1978).

Antiviral compounds. 9-(2-Hydroxyethoxymethyl)guanosine (Acyclovir) and 9-(1,3-dihydroxy-2-propoxy-methyl)guanosine (DHPG), also known as 2' nor-dihydroxyguanosine, Biolf-62 or BW759, was a gift from Dr P. Collins, Wellcome Foundation Ltd., Beckenham, Kent, U.K. (E)-5-(2-Bromovinyl)-2'-deoxyuridine (BVdU) was a gift from Professor E. de Clercq, Rega Institute, Leuven, Belgium. The drugs were dissolved in water and administered either in the drinking water or by intraperitoneal inoculation in a volume of 0.1 or 0.2 ml. The activity of the compounds against HSV-1 was determined by the plaque reduction method using preformed monolayer cultures of BHK-21 cells (Field et al., 1979).

Histology. Animals were killed at intervals after inoculation. The brain, eyes, optic nerves and spinal cord were removed and fixed in buffered formol-saline for light microscopy. After processing and embedding in paraffin wax, 5 µm thick sections were stained with haematoxylin and eosin. In some cases the immunoperoxidase stain was employed to locate HSV antigens. The antisera used were Dakopatts HSV-1 (B114) raised in rabbit (Miles-Yeda Ltd.) and Miles code no. 61-242 peroxidase-antiperoxidase (rabbit). Uninfected tissue was included as a negative control. No staining of infected foci was observed when preimmune serum was used in this test.

DNA extraction. High molecular weight DNA was extracted from mouse neural tissues as follows. The tissue was homogenized in 10 mM-Tris-HCl, 50 mM-EDTA pH 8. SDS was added to a concentration of 0.5%. The homogenate was then treated with Pronase (1 mg/ml) at 37 °C for 6 h followed by two phenol/chloroform extractions. The DNA was ethanol-precipitated, spooled, allowed to dry and redissolved in 10 mM-Tris-HCl, 1 mM-EDTA pH 7.6. This final step was repeated.

Blot hybridizations. High molecular weight DNA extracted from acute or DHPG-treated HSV-infected mouse brains or uninfected mouse brain was cleaved with an excess of BamHI. Following gel electrophoresis in 0.8% agarose, DNA was transferred to a nitrocellulose filter (Southern, 1975). The HSV-1 BamHI K fragment was labelled in vitro with [α-32P]dCTP by nick translation (Rigby et al., 1977) to a specific activity of 5 x 107 cts/min/µg.

The nitrocellulose filter was pre-hybridized for 7 h at 70 °C in 6 x SSC (1 x SSC = 0.15 M-sodium chloride, 0.015 M-sodium citrate), 0.5% SDS, 100 µg/ml calf thymus (CT) DNA, 2 x Denhardt’s solution (0.04% Ficol, 0.04% polyvinylpyrrolidone, 0.04% bovine serum albumin) and 10 mM-phosphate. Hybridization was performed at 70 °C in 4 x SSC, 0.5% SDS, 100 µg/ml CT DNA, 3 x Denhardt's solution, 10 mM-phosphate and 10% dextran sulphate. Washes were performed in 1 x Denhardt’s solution, 6 x SSC, 10 µg/ml CT DNA, 10 mM-phosphate followed by 1 x Denhardt’s solution, 1 x SSC, 10 µg/ml CT DNA, 10 mM-phosphate and finally 0.1 x SSC and 0.5% SDS at 70 °C. Autoradiography was carried out using Kodak X-Omat S film and light-intensifying screens for an exposure of 1 to 5 days.

RESULTS

Intranasal inoculation of adult BALB/c mice with a relatively high dose of HSV-1 (strain SC16) produced an encephalitis. The inoculum required to produce an LD50 was approx. 105 p.f.u. In all experiments a tenfold higher dose was employed in order to produce a universally fatal disease. After inoculation, mice appeared clinically normal for 3 days. On the 4th or 5th day
they became 'ruffled' and 'hunched' in appearance and the animals rapidly progressed to death. None survived beyond the 7th day after inoculation.

On the 3rd day antigen-containing cells were invariably present in the trigeminal ganglion and in the brain stem. By 5 days florid bilateral brain stem involvement was a consistent finding (Fig. 1a, b). The olfactory lobes were also frequently involved at 5 days, and between 5 and 7

Fig. 1. Untreated mouse 6 days after intranasal inoculation with HSV. (a) Virus antigen in neurons and glial cells of the brain stem. Positive cells are very dark, as the reaction product is brown. In many cells the staining is most intense in the nucleus. The proximal parts of some neuronal dendrites and axons are also stained (arrowed). Immunoperoxidase, × 360. (b) Bilateral distribution of antigen-positive cells in the brain stem (arrowed). The band of small dark nuclei in the cerebellum is the granule cell layer (GC). No brown reaction product was present in this layer, which only appears dark in the photograph because the cells are compact and have dense nuclear chromatin. Immunoperoxidase, × 22.
days the virus spread to variably infect the hippocampus, amygdala and parts of the cerebral cortex.

The CNS was also examined for the presence of infectious virus. The brain was separated into several parts and the amount of infectivity present in each was tested independently. By day 3 evidence of extensive virus replication was found in the trigeminal ganglion, brain stem and olfactory lobe (Table 1) and by day 5 after inoculation all samples contained high titres of infectious virus, particularly the trigeminal ganglion (a relatively small sample), brain stem and eye. Histological examination of the eye showed the anterior parts were involved, with lesions in the ciliary body and cornea. However there was no sign of infection spreading via the optic nerve, and the retina was spared. The important point drawn from these preliminary studies was that by day 3 after inoculation an active and progressive CNS infection was established in all mice, this being 1 or 2 days before clinical signs appeared.

**Effects of Acyclovir on intranasally infected mice**

The addition of ACV to the drinking water had previously been shown to be an extremely effective means of chemotherapy in mice given a non-lethal infection in a skin site (Field & de Clercq, 1981; Field & Neden, 1982). Mice inoculated intranasally were therefore provided with drinking water containing ACV (1 mg/ml). Therapy was commenced at various times after inoculation and mice were examined twice daily for clinical signs and death. It may be seen in Fig. 2 that mice were fully protected from lethal disease provided that therapy commenced at the same time as virus inoculation. When the start of treatment was withheld to day 2 or later all mice developed clinical signs and some died; in some cases death followed a more protracted disease. No protection was observed when treatment was started on day 3 or later. However, it was likely that mice developing encephalitis reduced their frequency of drinking: thus, their drug intake almost certainly declined. For this reason further experiments were carried out in which ACV was administered by intraperitoneal (i.p.) inoculation twice daily. In all subsequent experiments chemotherapy was started at 3 days after virus inoculation. The results showed that there was significant protection in that death was delayed but the majority of mice died after a protracted disease. A more frequent dose schedule was employed in case the 12 h interval between ACV infections might lead to significant periods of suboptimal ACV concentration in the CNS and that this might influence the outcome of the disease. When the same daily dose of ACV (50 mg/kg/day) was given either 6-hourly or 12-hourly the results demonstrated that the drug was clearly more effective when given with the shorter intervals between doses (Fig. 3).

**Effect of Acyclovir on virus replication in the CNS**

Having shown a gross effect of ACV on the outcome of infection as judged by survival of mice, the results of therapy were examined in more detail by examining virus replication in several different tissues involved in the infection. The diagram (Fig. 4) portrays the virus

### Table 1. Virus titres in neural tissues following intranasal inoculation*

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Day 3</th>
<th>Day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eyes†</td>
<td>1.4 ± 0.8</td>
<td>4.6 ± 0.9</td>
</tr>
<tr>
<td>Cerebrum</td>
<td>§</td>
<td>2.9 ± 1.5</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>1.8 ± 0.8</td>
<td>4.4 ± 1.0</td>
</tr>
<tr>
<td>Brain stem</td>
<td>3.2 ± 1.0</td>
<td>5.3 ± 0.6</td>
</tr>
<tr>
<td>Olfactory lobes</td>
<td>3.2 ± 1.0</td>
<td>3.4 ± 1.0</td>
</tr>
<tr>
<td>Trigeminal ganglia</td>
<td>3.2 ± 1.0</td>
<td>4.4 ± 0.5</td>
</tr>
</tbody>
</table>

* Mice were inoculated with 10⁶ p.f.u. HSV-1 SC16.
† Geometric mean value from five mice.
‡ Anterior eye, not retina or optic nerve.
§ 1/5 mice only yielded virus in the cerebellum (titre 2.5).
Herpes encephalitis chemotherapy

Fig. 2. Cumulative frequency of death in ACV-treated mice inoculated intranasally with HSV. Groups of 10 mice were inoculated intranasally (10⁶ p.f.u.) with HSV-1 strain SC16 and treated with ACV dissolved in the drinking water (1 mg/ml) commencing at various times after inoculation as follows: ○, control, untreated; ○, ACV in drinking water, therapy commenced at day 4 post-infection; ▲, ACV in drinking water, therapy commenced at day 3 post-infection; △, ACV in drinking water, therapy commenced at day 2 post-infection; □, ACV in drinking water, therapy commenced at day 1 post-infection; ■, ACV in drinking water, therapy commenced from time of virus inoculation.

Fig. 3. Cumulative frequency of death in mice inoculated intranasally with HSV-1 SC 16 (10⁶ p.f.u.) and treated by injecting a solution of ACV i.p. at a dose of 50 mg/kg/day starting on the 3rd day after infection. ○, Control, untreated; ○, ACV, 12-hourly doses; ▲, ACV, 6-hourly doses.

Infectivity titres observed in the different neural samples obtained from individual mice with or without therapy at various times after inoculation. All mice had evidence of active virus replication in the CNS at the time of commencing therapy and for a period thereafter. No untreated mice survived beyond the 8th day and therefore no tissues were obtained at later times from this group. Virus titres stabilized or declined in ACV-treated mice although half the samples contained small or moderate titres of virus on day 7 after 4 days of therapy and one of two mice contained low levels of infectious virus in the trigeminal ganglion, brain stem and cerebellum 10 days after inoculation. No residual virus was found in the second mouse sample at this time or in three mice examined at day 10 in a subsequent experiment (see below and Fig. 7). All mice initially showed signs of disease but those surviving beyond the 10th day became clinically normal with shiny fur and appeared to resume their increase of size and weight.

Histological examination supported these results. Between 3 and 7 days control animals all showed increasing density of antigen-positive cells in affected regions of the CNS and
Fig. 4. Diagrammatic representation of the distribution of virus in the brains of individual mice infected intranasally with $10^6$ p.f.u. HSV. Chemotherapy (50 mg/kg/day) given i.p. in 6-hourly doses commenced 3 days after virus inoculation.

terminally a wide distribution in the brain stem and cerebral hemispheres. In ACV-treated animals the numbers of antigen-positive cells identified at 5 days was comparable to that of controls, but by 7 days there was a clear reduction. In the majority of animals virus antigen was no longer detectable in the trigeminal ganglion. Small groups of antigen-containing cells remained in the brain stem (Fig. 5a, b) and there were occasional positive cells in the hypothalamus. At 10 days, only isolated, positive cells were present in the brain stem of 50% of surviving mice. In the remainder no virus antigen could be detected (Fig. 6), although foci of lymphocytes and myelin breakdown provided clear evidence of recent active infection.

Comparison of three potent nucleoside analogues

Three different nucleoside analogues were then tested under identical conditions in the model described below. The compounds for comparison with ACV were BVdU and DHPG. All three drugs were found to be potent inhibitors of HSV-1 SC16 when tested in BHK-21 cell monolayers using the plaque reduction test. The values for ED$_{50}$ were found to be 0.02 μg/ml with ACV, 0.01 μg/ml with BVdU and 0.1 μg/ml with DHPG. Each drug was given to mice at a total dose of 50 mg/kg/day by i.p. injection at 6 h intervals to groups of 10 animals starting from the third day after intranasal inoculation of virus; one group of mice was left untreated. At regular intervals after inoculation two mice from each group were killed and virus titres were measured in the various parts of the CNS as before.

No control, untreated mice survived beyond the 6th day. BVdU therapy appeared to increase survival time slightly but no mice in this group survived beyond the 8th day after inoculation and the two BVdU-treated mice examined on day 7 yielded high titres of infectious virus from all specimens examined (Fig. 7). Histological examination of BVdU-treated mice at 6 to 8 days showed numerous antigen-positive cells in the same distribution and approaching a similar density to that observed in controls (Fig. 8). The results of virus titrations on ACV-treated mice were similar to those obtained in the previous experiment except that no virus was detected in the two mice tested on day 10. The DHPG-treated mice were notable in that the mice showed no obvious clinical signs and appeared healthy throughout the experiment. Furthermore, only low levels of infectious virus were detected in mice after therapy commenced, the first mice being examined at day 5, 2 days after treatment started (Fig. 7). DHPG-treated mice examined by histology at 7 days had very few antigen-positive cells in the brain stem.

Attempts to detect latent HSV in mice following chemotherapy

Mice that had recovered following 6-hourly chemotherapy with either ACV or DHPG were tested for the presence of latent HSV. Although virus could not always be recovered from
trigeminal ganglia following 6 days incubation of the explanted tissue, the drug was not found to prevent the establishment of latency and in one case all eight of eight pairs of trigeminal ganglia from DHPG-treated mice yielded virus on reactivation several weeks after recovery from the infection. However, the eyes, olfactory lobes and brain stems from the recovered mice were also tested but none of these samples ever yielded virus on attempted reactivation. These negative results should not be overemphasized; while this reactivation procedure proved effective in

Fig. 5. ACV-treated mouse at 7 days. (a) Scanty virus antigen-positive cells in the brain stem (arrowed). Compare with Fig. 1 (b). Immunoperoxidase, × 22. (b) The same region as in (a) at a higher magnification. The virus antigen-positive cells (VA) are associated with myelin breakdown, shown by vacuolation (M) and adjacent small blood vessels show lymphocytic cuffing (L). Immunoperoxidase, × 112.
Fig. 6. ACV-treated mouse at 10 days showing complete absence of antigen-positive cells in the brain stem, although a small focus of chronic inflammatory cells is present (arrowed). (Large irregular specks are artefacts.) Immunoperoxidase, × 22.

Fig. 7. Diagrammatic representation of the distribution of virus in mice infected intranasally with HSV (10⁶ p.f.u.), comparing three different nucleoside analogues. Therapy was started at 3 days after virus inoculation. In each case the drug was given i.p. (50 mg/kg/day) in 6-hourly doses.
detecting latency in ganglia after peripheral inoculation, it could be less well suited to the analysis of CNS tissue.

Clearance of virus from CNS of DHPG-treated mice

The experiments above on intranasally infected mice describe the successful chemotherapy, using DHPG, of herpes encephalitis with treatment commencing after virus replication was established in various parts of the brain. Virus (as detected by infectivity titration on homogenized tissues) was cleared surprisingly quickly in DHPG-treated mice. This raised the question as to whether significant amounts of non-replicating virus remained in neural tissue following suppression of active virus replication by the inhibitor. It was not possible to examine sufficient histological sections to determine precisely the average time of disappearance of virus from the brain but by day 7 almost all virus antigen appeared to have been eradicated except for sparse positive cells in the brain stem and at later times none was detected. Particular attention was paid to the trigeminal ganglion, brain stem and eye but no trace of virus was observed.

The recent report of Rock & Fraser (1983) that HSV genomes were detected by means of Southern blotting in the DNA obtained from the trigeminal ganglia and brain stems of mice that survived a sublethal encephalitis after ocular inoculation prompted us to apply similar techniques to neural tissues obtained from our mice which had survived following DHPG chemotherapy. The following samples were examined: trigeminal ganglia (pooled from three mice), brain stem and olfactory lobes from mice on day 5 after inoculation and on day 10 (after 7 days chemotherapy). In addition, similar samples were obtained from uninfected mice and also a reconstruction at 0·1 and 1 genome copy per cell were prepared by mixing purified virion DNA with uninfected mouse brain. The DNA was digested using the restriction enzyme BamHI and a labelled probe was used which comprised the cloned BamHI K fragment of HSV-1. This clone hybridizes to the junction region of the HSV-1 genome and therefore recognizes three bands of BamHI-digested HSV-1 DNA corresponding to the junction region K and both terminal fragments P and S which contain the inverted repeated sequences (Fig. 9).
Fig. 9. Detection of fragments of HSV-1 DNA in mouse nervous tissue following intranasal inoculation. Ten µg of high molecular weight DNA was digested with BamHI, transferred to nitrocellulose and hybridized to a 3²P-labelled BamHI K probe. Lanes 1, 2 and 3 are brain stem, pooled trigeminal ganglia and olfactory lobe DNA respectively, sampled 5 days post-infection. Lanes 4, 5 and 6 are brain stem, trigeminal ganglia and olfactory lobe DNA respectively, sampled 10 days post-infection (DHPG therapy having been initiated at 3 days post-infection). Lane 7 is 10 µg of whole brain DNA taken from uninfected control animals. Lanes 8 and 9 are 0·1 and 1 copy/cell reconstructions respectively, using HSV-1 strain F DNA extracted from purified extracellular virions.

In the samples examined on day 5 (untreated mice) bands are clearly visible which correspond to the infectious virus measured in further mice inoculated at the same time (4·8 ± 0·1, 2·8 ± 1·2 and 2·4 ± 0·5 log₁₀ p.f.u. in brain stem, trigeminal ganglia and olfactory lobes respectively). Further virus-specific lines between the major bands probably result from reiterated sequences known to be a feature of HSV DNA (Wagner & Summers, 1978). Inspection of the gel tracks loaded with DNA obtained 10 days after inoculation revealed no evidence of virus DNA. The reconstruction suggests that 0·1 genome copy per cell would have been readily detected. Longer exposure of the gels revealed a number of faint cross-reactive bands (also present in uninfected DNA), two of which coincided with the HSV junction region bands. This cross-hybridization with cellular DNA would make the detection of viral sequences present at a low copy number difficult. However, the result of this experiment strongly suggests that large amounts of HSV DNA do not remain in the infected cells after the disappearance of infectious virus and antigens from the tissue.

**DISCUSSION**

There have been many previous reports of the use of mouse models for herpes encephalitis to evaluate potential antiherpetic drugs. The present study differs from most preceding ones in that virus replication was monitored in different parts of the brain both before and during chemotherapy. The most common method for inducing herpes encephalitis employs direct intracerebral inoculation, but this has the disadvantage of being a less natural way of introducing virus because it may for example create cerebral trauma and produce a gross increase in intracranial pressure (Mims, 1960). For this study the intranasal route was chosen because it appeared to give a reproducible disease which was analogous to human encephalitis. In mice the
distribution of virus antigen in the cerebrum was similar to that observed in many fatal human cases (Esiri, 1982). Herpetic brain stem encephalitis, although relatively unusual, has also been described in man (Dayan et al., 1972).

A consequence of using a non-neural route of virus inoculation for studying the therapy of encephalitis (for example intraperitoneal, intravenous or intranasal) is that the agent may inhibit the virus while it is replicating in non-neural tissues and thus may act by preventing the initiation of CNS disease. This might in part, explain our observation that therapy using oral ACV was extremely effective when commenced within 2 days of virus inoculation but not when treatment was started later. Similar protection by early therapy was reported by De Clercq et al. (1979) using BVdU after intranasal inoculation. However, Park et al. (1979) and Schinazi et al. (1983) investigating ACV and fluoroarabinosylodocytosine respectively also obtained protection by use of early therapy and in these cases virus was inoculated intracerebrally and therefore almost certainly must have involved inhibition of virus replication within the CNS. In the present study we showed conclusively that virus titres were reduced and fatal disease could be prevented by i.p. therapy with either ACV or DHPG even when drug treatment was started after virus replication was established in all major compartments of the brain.

Our most important observation regarding ACV was that therapy was markedly less effective when dose intervals were 12 h compared with 6 h. This is probably because drug levels achieved in CNS are relatively low, approx. tenfold lower than those achieved in serum (De Miranda et al., 1982). There is one report which showed that a single subcutaneous dose of ACV (100 mg/kg) achieved a drug concentration in the CNS of approx. 30 μM falling to 10 μM (Biron et al., 1982). However, the earlier study of Kern et al. (1982) suggested much lower levels; 60 mg/kg/day i.p. given 12-hourly gave brain levels of only 4.2 to 8.2 μM 1 h after drug inoculation and this rapidly declined to 0.2 μM after 2 h and < 0.05 μM after 3 h. These data would adequately explain our finding of lower efficiency obtained with 12 h interval between drug doses. In the same study (Kern et al., 1982) it was also shown that oral dosing by means of the drinking water (1.5 mg/ml) regularly maintained drug levels in the CNS of 5 to 10 μM. This continuity of the presence of the inhibitor would explain why this method of oral dosing was very effective. Our failure to protect mice by ACV in the drinking water when therapy was withheld until the third day was probably partly because mice began to stop drinking with the onset of neural disease.

When the intranasal encephalitis model was used to compare three nucleoside analogues, BVdU was found to be much the least effective. This is despite BVdU having almost tenfold better activity than ACV against the same virus when tested in tissue culture. Others have shown BVdU to be highly efficacious against HSV encephalitis in mice though generally using higher doses of drug (e.g. Reeschlager et al., 1982). However, in our own previous studies in BALB/c mice, we found BVdU to be poorly effective in the treatment of skin infections (Field & de Clercq, 1981; Field & Neden, 1982). We suggest three possible reasons for the poor performance of BVdU. First, the compound is readily converted in mice to bromovinyluracil (which is inactive) by phosphorylase attack (I. Sim, personal communication). Second, thymidine levels in inflamed tissue such as is found in acute HSV infection are likely to be high. The inhibition of HSV by BVdU is more sensitive to thymidine than either ACV or DHPG since the triphosphates of the latter compounds compete with dGTP rather than TTP for the HSV DNA polymerase (Larsson et al., 1983). Finally, the distribution of BVdU to the CNS in mice is thought to be less efficient (de Clercq et al., 1982).

Of the three drugs tested, DHPG appeared to be the most effective and clearly warrants further investigation as a potential therapeutic agent for herpes encephalitis. DHPG showed similar activity against HSV when tested in vitro and was not markedly superior to ACV when compared in an established skin infection in mice (our unpublished observations). Pharmacological data on DHPG in the mouse are not yet available but the most likely reasons contributing to its superiority are that the compound passes more efficiently through the blood–brain barrier and that phosphorylation of the drug is relatively more efficient in mouse cells.

Following DHPG therapy the clearance of virus from infected mice was remarkably complete. No infectious virus remained after 7 days of therapy and no mice developed clinical signs suggesting relapse when therapy was terminated at this time; all mice surviving at 10 days
post-infection were long-term survivors. No virus antigens were detectable in the brains of recovered animals even in areas showing histological signs of residual tissue damage. Virus DNA could not be detected in the brains of these mice using molecular hybridization techniques. We have no evidence that the brains of recovered mice contain large amounts of 'latent' herpes DNA as described recently by Rock & Fraser (1983) in the brain stems of mice that had recovered from a sublethal encephalitis after ocular inoculation. Clearly our negative findings require much further investigation before any conclusions may be drawn about the effects of nucleoside analogues on the establishment of 'latent' infection in the CNS. The question of whether or not true latent HSV can be established in the CNS either spontaneously or under the influence of nucleoside analogues is a question of both fundamental and practical interest and one which we will continue to pursue.

When effective antiviral chemotherapy for encephalitis is employed in man we must expect that some patients who would otherwise have died from the disease will survive with neurological sequelae. Our mouse model is well suited to the study of long-term histopathological changes in the brains of animals that have been rescued from a potentially fatal encephalitis. The pathogenesis of HSV-induced neural damage in surviving mice is now the subject of further investigation.

H. J. F. is funded by a grant from the Medical Research Council. J. R. A. is in receipt of a grant from the East Anglian Regional Health Authority. S. E. holds a CASE award supported by The Wellcome Foundation Ltd. We wish to thank Mrs Elizabeth Lay and Mrs Beverley Wilson for their expert technical assistance, Mrs Mary Wright for preparing the manuscript and Mr Christopher Burton for photography.

REFERENCES


*(Received 19 October 1983)*