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Antiviral Research 45 (2000) 157–167

Antiviral Research

www.elsevier.com/locate/antiviral

Antiviral activity of ganciclovir elaidic acid ester against herpesviruses

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Received 19 August 1999; accepted 5 January 2000

Abstract

A fatty acid derivative of ganciclovir (GCV), elaidic acid ganciclovir (E-GCV), has been evaluated for its inhibitory activity against laboratory and clinical strains of herpes simplex type 1 (HSV-1) and type 2 (HSV-2), varicella-zoster virus (VZV) and human cytomegalovirus (HCMV) in human embryonic lung fibroblasts. GCV, cidofovir, acyclovir (ACV), brivudin (BVDU) and foscarnet (PFA) were included as reference compounds. The viruses studied were wild-type, thymidine kinase-deficient (TK^-) and PFA-resistant (PFA^r) HSV strains. The IC_{50} values obtained for E-GCV were 5- to 30-fold lower than those observed for GCV, the IC_{50} value of E-GCV for HSV-1 strain KOS being 0.07 nM. A similarly increased activity of E-GCV (as compared to GCV) was noted for TK^- and PFA^r HSV-1 or HSV-2 strains. However, E-GCV did not exhibit superior activity over GCV to VZV or HCMV in vitro. The antiviral efficacy of E-GCV was also evaluated in vivo against intracerebral HSV-2 infection in NMRI mice. Animals were treated intraperitoneally or perorally with E-GCV, GCV or placebo once daily for 10 days, starting the day of infection. E-GCV compared to GCV at equimolar doses, proved markedly more efficacious than GCV in terms of reduction of mortality rate and delay of mean time of death. The elaidic acid ester of GCV should therefore be considered as a novel approach towards the treatment of HSV infections.

Keywords: HSV; Ganciclovir elaidic ester; Prodrug

1. Introduction

Herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2), varicella-zoster virus (VZV) and human cytomegalovirus (HCMV) cause diseases which are usually self-limiting in the immunocompetent host (Corey and Spear, 1986); however, they can be a major cause of morbidity and mortality in the immunocompromised population. HSV and VZV are mostly responsible for mucocutaneous infections (Safrin et al., 1991; Weller, 1992; Wutlzer, 1997). HCMV is usually associated with

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0166-3542/00/$ - see front matter
PII: S0166-3542(00)00070-X

interstitial pneumonia in bone marrow and solid organ transplant recipients while retinitis is the most common manifestation of HCMV infection in AIDS patients (Alford and Britt, 1993). Several agents have been developed for the treatment of herpesvirus infections. One of these, ganciclovir (GCV) (9-(1,3-dihydroxy-2-propoxymethyl)guanine, DHPG), a homologue of acyclovir (ACV), is the first antiviral drug that proved to be efficacious in the treatment of CMV disease in humans (Ashton et al., 1982; Martin et al., 1983). GCV not only inhibits CMV but also other herpesviruses. Thus, in cell culture, GCV has excellent activity against HSV-1, HSV-2, VZV (Smith et al., 1982; Field et al., 1983; Crumpacker et al., 1984), Epstein-Barr virus (EBV; Lin et al., 1984) and human herpesvirus-6 (HHV-6; Agut et al., 1989). GCV inhibits viral DNA polymerases, including those of HSV, VZV and CMV, by competitively interfering with the incorporation of deoxyguanosine triphosphate into the elongating viral DNA (Field et al., 1983; Cheng et al., 1983a). Drug activity requires conversion to the triphosphate derivative. In cells infected with HSV and VZV, the viral thymidine kinase (TK) phosphorylates GCV to the monophosphate form, and cellular enzymes further convert the monophosphate to the triphosphate form, which then inhibits viral DNA synthesis. CMV, however, does not encode for a specific TK. Instead, the CMV genome encodes for a protein kinase, the product of the CMV UL97 gene, which is able to phosphorylate GCV (Littler et al., 1992; Sullivan et al., 1992). Unlike ACV, GCV does not act as an obligatory chain terminator following incorporation into DNA; and internal incorporation of GCV into DNA has been reported (Cheng et al., 1983b; Frank et al., 1984).

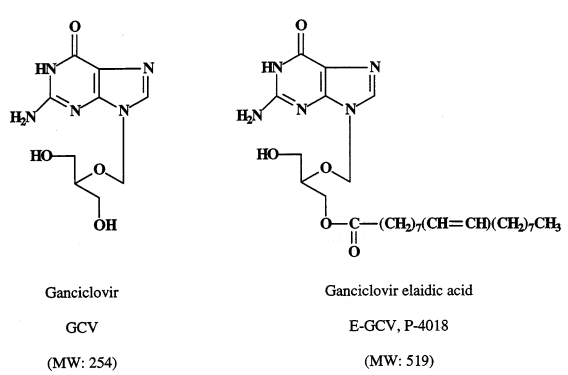

Fig. 1. Formulae of GCV and ganciclovir elaidic acid (E-GCV, P-4018).

Intravenous GCV has proved effective in the treatment of several types of CMV infection, including CMV retinitis in AIDS patients, CMV infections of the gastrointestinal tract and central nervous system, and interstial CMV pneumonia (Crumpacker, 1996; Noble and Faulds, 1998). GCV is also used for maintenance therapy and prophylaxis. Prolonged maintenance therapy with suboptimal doses and frequent discontinuation due to the toxic effects of the drug favor the emergence of GCV-resistant (GCV^r) HCMV strains (Drew et al., 1991). The isolation of GCV^r HCMV strains has been reported with increasing frequency and is a matter of major concern (Erice et al., 1989; Stanat et al., 1991). Oral GCV has been approved as an alternative to intravenous maintenance therapy for CMV retinitis in AIDS patients. In addition, oral GCV has proved effective in the prevention of CMV disease after solid organ transplantation. In man, the oral bioavailability of GCV is low, only 6–9%. Thus, it would seem imperative to search for an oral prodrug of GCV that may result in increased bioavailability.

In addition, GCV has been found to be cytostatic against a variety of tumor cell lines transfected with the HSV-1 or HSV-2 TK gene (Moolten, 1986; Borelli et al., 1988; Balzarini et al., 1994). Several reports have documented the usefulness of GCV in the combined gene/chemotherapy of malignant brain tumors (Culver et al., 1994; Raffel et al., 1994; Kun et al., 1995).

In this study we describe the in vitro antiviral activity of the elaidic acid ester prodrug derivative of GCV (designated E-GCV) (Norsk Hydro, Porsgrunn, Norway) (Fig. 1) against HSV, VZV and HCMV. The antiviral efficacy of E-GCV, compared to that of GCV, was also evaluated in an in vivo model of intracerebral HSV-2 infection in mice.

2. Materials and methods

2.1. Cells

Human embryonic lung (HEL) fibroblasts (ATCC CCL137) were used. Cells were propagated in minimum essential medium supplemented with 10% inactivated calf serum (Integro, The Netherlands), 1% L-glutamine and 0.3% sodium bicarbonate.

2.2. Viruses

The following reference (laboratory) strains were used: HCMV strains AD169 and Davis, HSV-1 strain KOS, TK^- HSV-1 strain B2006, HSV-2 strains G and 196, VZV strains Oka and YS, and TK^- VZV strains YS-R and 07-1. The clinical HSV-1 strains Hu-3, Hu-5 and Hu-10 were isolated from a bone marrow transplant recipient and the clinical HSV-2 strains HS-44 and HS-47 were recovered from an AIDS patient (Snoeck et al., 1994).

2.3. Drugs

The origin of the antiherpetic compounds used was as follows: acyclovir (ACV, 9-(2-hydroxyethoxymethyl)guanine), Wellcome Research Laboratories, Research Triangle Park, NC; foscarnet (phosphonoformate, PFA), Sigma, St. Louis, MO; HPMPC (cidofovir, (*S*)-1-(3-hydroxy - 2 - phosphonylmethoxypropyl)cytosine), Gilead Sciences, Foster City, CA; brivudin (BVDU, (*E*)-5-(2-bromovinyl)-1-(β-D-2-deoxyribofuranos-1-yl-uracil), the Rega Institute for Medical Research, Leuven, Belgium; ganciclovir (GCV, 9-(1,3-dihydroxy-2-propoxymethyl)-guanine, DHPG) and ganciclovir-elaidic acid ester (E-GCV), Norsk Hydro, Porsgrunn, Norway.

2.4. Antiviral assays

Cells grown in 96-well microtiter plates were inoculated with virus at an input of 100 plaque-forming units (PFU) (HCMV), 20 PFU (VZV) or 100 $CCID_{50}$ (HSV-1 and HSV-2), one $CCID_{50}$ representing the virus dose that is infective for 50% of the cultures. Varying concentrations of the test compounds were added in duplicate after virus adsorption for 2 h at 37°C. After 2–3 days (HSV-1 and HSV-2), 5 days (VZV) or 7 days (HCMV) of incubation at 37°C in 5% CO_2 atmosphere, virus-induced cytopathicity (HSV and HCMV) or virus plaque formation (VZV) was determined and the 50% inhibitory concentration (IC_{50}) was determined as the compound concentration required to reduce virus-induced cytopathicity or viral plaque formation by 50%.

2.5. Cytotoxicity assays

Toxicity of the compounds for the host cells was based on inhibition of cell growth. The cells were seeded at 4×10^3 cells per well into 96-well microtiter plates and allowed to proliferate for 24 h in minimum essential medium (MEM) containing 20% FCS. Twenty-four hours later, MEM containing different concentrations (in duplicate) of the test compounds was added. After 3 days of incubation at 37°C, the cell number was determined with a Coulter counter. The minimum cytotoxic concentration is expressed as the CC_{50} (50% cytotoxic concentration), or concentration required to reduce cell growth by 50% relative to the number of cells in the control cultures. The minimum cytotoxic concentration (MCC), or compound concentration required to cause a microscopically visible alteration of normal cell morphology was also determined.

2.6. Virus yield assays

Confluent HEL cells grown in 24-well microtiter plates were infected with HSV-1 (strain KOS) or HSV-2 (strain G) at a multiplicity of infection of approximately 0.1. At 2 h, residual virus was renewed and replaced by medium containing different concentrations of the test compounds. After 3 days (for HSV-1) or 4 days (for HSV-2) post-infection, the cell supernatants were harvested and frozen at −80°C until virus titration. The supernatants were assayed for virus yield by plaque formation in HEL cells.

Table 1
Activity of E-GCV against different HSV strains in HEL cells[a]

Compound	IC_{50} (μM)[b]			CC_{50} (μM)[c]	MCC (μM)[d]
	HSV-1 (KOS strain)	HSV-2 (G strain)	HSV-1 TK^- (B2006 strain)		
E-GCV	0.001 ± 0.001	0.0012 ± 0.0016	>10	135 ± 92	40 ± 0
Elaidic acid	>100	>100	>100	150 ± 0	100 ± 0
GCV	0.007 ± 0.004	0.016 ± 0.017	16	230 ± 42	>254
ACV	0.021 ± 0.005	0.031 ± 0.011	120	>844	>844
BVDU	0.015 ± 0.009	>300	>601	>601	>601

[a] Mean values for two separate experiments.
[b] Concentration required to reduce virus-induced cytopathicity by 50%.
[c] Concentration required to reduce cell growth by 50%.
[d] Minimum cytotoxic concentration, or compound concentration required to cause a microscopically visible alteration of normal cell morphology

2.7. In vivo experiments: intraperitoneal and peroral treatment of intracerebral HSV-2 infection in NMRI mice

Twenty-five-day-old NMRI mice (weighing 11–13 g) were inoculated intracerebrally with HSV-2 (196 strains) at 0.3 $CCID_{50}$/0.02 ml per mouse and treated either intraperitoneally (i.p.) or perorally (via gavage) with the indicated doses of GCV or E-GCV once daily for 10 days, starting on the day of virus infection. Ten mice per group were used. Mortality of mice was recorded till day 20 after virus inoculation. Statistics significance of survival was assessed by the χ^2-test with Yates' correction, and the differences in the mean day of death were evaluated using the two-tailed Student's t-test. A P value of 0.05 or less was considered significant.

3. Results

3.1. Activity of E-GCV against herpes simplex virus

GCV and E-GCV were evaluated for their inhibitory activities against laboratory and clinical strains of HSV-1 and HSV-2 in HEL cells. GCV inhibited the replication of the HSV-1 and HSV-2 laboratory strains at an IC_{50} of 0.007 and 0.016 μM, respectively (Table 1). IC_{50} values for E-GCV were about seven- to eight-fold lower than those observed for GCV. The free fatty acid elaidic acid did not show any activity by itself up to the highest concentration tested (100 μM). ACV inhibited the HSV-1 and HSV-2 laboratory strains at similar IC_{50} values as GCV. The other reference compound included in the study, BVDU, showed an antiviral activity against HSV-1 that was comparable to that of GCV and ACV, while it was virtually inactive against HSV-2. BVDU depends for its second phosphorylation step on the dTMP kinase associated with the HSV-1 TK. The HSV-2 TK does not possess dTMP kinase activity which explains the lack of activity of BVDU against HSV-2 (Descamps and De Clercq, 1981). As expected, GCV, E-GCV, ACV and BVDU were less inhibitory to the replication of the TK^- HSV-1 strain B2006 than to that of the wild-type strain KOS.

Similar patterns of sensitivity or resistance to GCV and E-GCV were observed with clinical HSV isolates including ACV^r and ACV^r/PFA^r HSV-1 strains recovered from a bone marrow transplant recipient that had received multiple courses of ACV and PFA treatment (Table 2). Thus, ACV, BVDU, GCV and E-GCV were not active against the TK^- isolate (Hu-10); this strain was also resistant to PFA, which indicates that besides a mutation in the TK gene it also bears a mutation in the DNA polymerase gene. The Hu-5 strain appeared to have been mutated in the DNA polymerase gene, since it was resistant to the pyrophosphate analogue PFA but sensitive to the

Table 2
Activity of E-GCV against clinical HSV-1 and HSV-2 isolates in HEL cells[a]

Compound	IC_{50} (μM)[b]				
	HSV-1			HSV-2	
	Hu-3	Hu-5	Hu-10	HS-44 (TK^-)	HS-47 (TK^+)
E-GCV	0.0002 ± 0.0001	0.0005 ± 0.0001	$>100 \pm 0$	70 ± 42	0.0006 ± 0.0005
Elaidic acid	$>100 \pm 0$	$>100 \pm 0$	$>100 \pm 0$	$>100 \pm 0$	$>100 \pm 0$
GCV	0.0028 ± 0.0001	0.014 ± 0.001	$>400 \pm 0$	57 ± 61	0.0056 ± 0.0060
ACV	0.025 ± 0.006	0.21 ± 0	$>210 \pm 0$	190 ± 29	0.037 ± 0.032
BVDU	0.042	0.023	150	$>150 \pm 0$	$>150 \pm 0$
PFA	150 ± 24	584 ± 118	$>667 \pm 0$	47	71
HPMPC	2.86	3.32	4.65	0.97	1.15

[a] Mean values for two separate experiments.
[b] Concentration required to reduce virus-induced cytopathicity by 50%.

TK-dependent drugs, BVDU, GCV and E-GCV (although a ten-fold decrease in the sensitivity to ACV was noted). This is in agreement with previously reported results showing that PFA^r viruses are cross-resistant to ACV (Snoeck et al., 1994; Andrei et al., 1995). E-GCV proved to be 13- to 28-fold more active than GCV against the wild-type (Hu-3) and the PFA^r (Hu-5) HSV-1 strains, respectively. The acyclic nucleoside phosphonate cidofovir (HPMPC) proved equally active to ACV^r and ACV^r/PFA^r strains. When GCV and E-GCV were tested against a pair of HSV-2 isolates recovered from an AIDS patient, again E-GCV proved to be about eight-fold more active than GCV against the wild-type strain HS-47, while considerably reduced activity was observed with any of the TK-dependent drugs against the TK^- strain HS-44.

The superior activity of E-GCV compared to GCV against HSV-1 and HSV-2 was confirmed by a virus yield reduction assay. At concentrations of 50, 5 and 0.5 μM, both E-GCV and GCV reduced virus yield by about five logs (HSV-2) or six logs (HSV-1; Fig. 2). When lower concentrations of GCV and E-GCV were evaluated, i.e. 0.005 and 0.0005 μM, the inhibition in virus yield obtained with E-GCV was approximately 1 log higher than that observed with GCV.

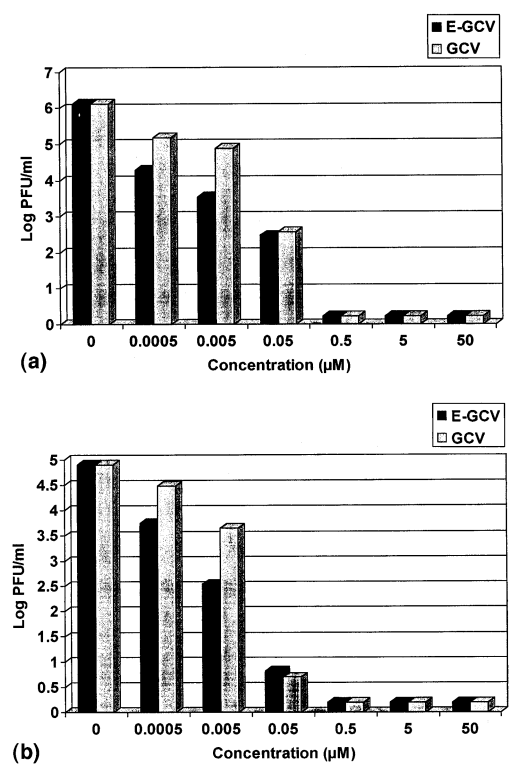

Fig. 2. Inhibitory effects of E-GCV and GCV on the yield of HSV-1, KOS strain (Panel A), and HSV-2, strain G (Panel B).

Table 3
Activity of E-GCV against HCMV and VZV in HEL cells[a]

Compound	IC_{50} (μM)[b]					
	HCMV		TK^+ VZV		TK^- VZV	
	AD-169 strain	Davis strain	Oka strain	YS strain	07-1 strain	YS-R strain
E-GCV	4.5 ± 0.7	3.5 ± 2.1	1.4 ± 0.8	4.5 ± 0.7	37 ± 19	38 ± 17
Elaidic acid	>100	>100	>100	>100	>100	>100
GCV	1.75 ± 0.3	3.8 ± 1.8	0.73 ± 0.68	2.6 ± 2.1	14 ± 2	24 ± 23
HPMPC	0.15 ± 0.04	0.21 ± 0.06	0.38 ± 0.26	0.58 ± 0.27	0.23 ± 0.10	0.08 ± 0.07
ACV	46	55	1.75 ± 1.70	2.47 ± 1.88	68 ± 42	93 ± 78
BVDU	>50	>50	0.0074 ± 0.0010	0.0047 ± 0.0047	>150	>150

[a] Mean values for two to three separate experiments.
[b] Concentration required to reduce virus-induced cytopathicity by 50%.

3.2. Activity of E-GCV against VZV and HCMV

When GCV and E-GCV were evaluated for their antiviral effect against VZV and HCMV reference strains, they proved equally active. No significant differences in the IC_{50} values of GCV and E-GCV were noted with the wild-type strains. Thus, GCV and E-GCV inhibited HCMV replication in HEL cell cultures at an IC_{50} of 2.28 and 4 μM (mean values for AD-169 and Davis strains), respectively (Table 3). The replication of VZV wild-type strains was inhibited at an IC_{50} of 1.67 and 2.95 μM (mean values for Oka and YS strains) by GCV and E-GCV, respectively (Table 3). Both GCV and E-GCV proved less active against the TK^- VZV strains.

E-GCV was about two times more inhibitory to HEL cell growth than GCV. Also, cell morphology was altered by E-GCV at a minimum cytotoxic concentration (MCC) of 40 μM, which was about six-fold higher than that of GCV. Elaidic acid inhibited cell proliferation and altered cell morphology from a concentration of 150 and 100 μM, respectively. These results suggest that the higher toxicity observed for E-GCV compared to GCV, may be due to the fatty acid moiety of the drug.

3.3. Intraperitoneal treatment of intracerebral HSV-2 infection in NMRI mice

Two independent experiments were performed to assess the efficacy of E-GCV compared to GCV in the intraperitoneal treatment of HSV-2 infection in NMRI mice (Fig. 3). Twice the dose (expressed in mg/kg) was administered for E-GCV as compared to GCV in order to maintain equimolar concentrations. Intracerebral inoculation of mice with HSV-2 resulted in 100% mortality after 6–7 days post-infection. In the first experiment (Fig. 3, Panel A), treatment with GCV did not significantly reduce the mortality rate when given up to doses of 10 and 20 mg/kg; however, a significant delay in the mean time of death ($P < 0.01$) was noted (about 2 days) at GCV doses of 20 and 10 mg/kg.

In marked contrast with GCV, E-GCV reduced the mortality from 100 to 30% when given at a dose of 10 or 20 mg/kg, and to only 10% when administered at a dose of 40 mg/kg. Even at a dose of 5 mg/kg E-GCV reduced the mortality to 40% after 20 days post-infection. Also a significant delay in the time of death was noted in the groups of mice treated with E-GCV ($P < 0.001$ for E-GCV at doses of 10, 20 and 40 mg/kg and $P < 0.05$ for E-GCV 5 mg/kg; Fig. 3, Panel A).

In the next series of experiments, lower doses of GCV and E-GCV were evaluated (Fig. 3, Panel B). Again, only 20% final mortality was noted in the group of mice treated with E-GCV at 10 mg/kg. At E-GCV doses of 2.5 and 5 mg/kg final mortality was 80%. At these doses, a significant delay in the time of death ($P < 0.05$) was observed

(mean time of death 8.63, 11.75 and 12.5 days for E-GCV 2.5, 5 and 10 mg/kg, respectively, compared to 6.2 days for the untreated control group). The corresponding doses of GCV (5, 2.5 and 1.25 mg/kg) had no influence on either the mortality rate or the mean time of death of HSV-infected mice.

3.4. Peroral treatment of intracerebral HSV-2 infection in NMRI mice

E-GCV was also compared with GCV in the peroral treatment of HSV-2 infection in NMRI mice (Fig. 3). In the first series of experiments (Fig. 4, Panel A), oral doses of GCV 80 and 160 mg/kg, as well as the corresponding equimolar doses of oral E-GCV, i.e. 160 and 320 mg/kg, respectively, resulted in complete protection against intracerebral HSV-2 infection. At doses of 20 and 40 mg/kg GCV reduced the mortality to 80 and 20%, respectively. Also at these doses a decrease in the mean time of death was noted, i.e. 10 days ($P < 0.05$) and 11.75 days ($P < 0.001$) for GCV doses of 40 and 20 mg/kg, respectively, compared to 5.1 days for the untreated mice. The corresponding equimolar doses of E-GCV proved more effective than GCV. Thus, treatment with E-GCV at 40 and 80 mg/kg resulted in only 40 and 10% mortality, with a mean time of death of 13 and 14 days ($P < 0.001$), respectively.

The superior activity of E-GCV over GCV was confirmed in the next experiment (Fig. 4, Panel

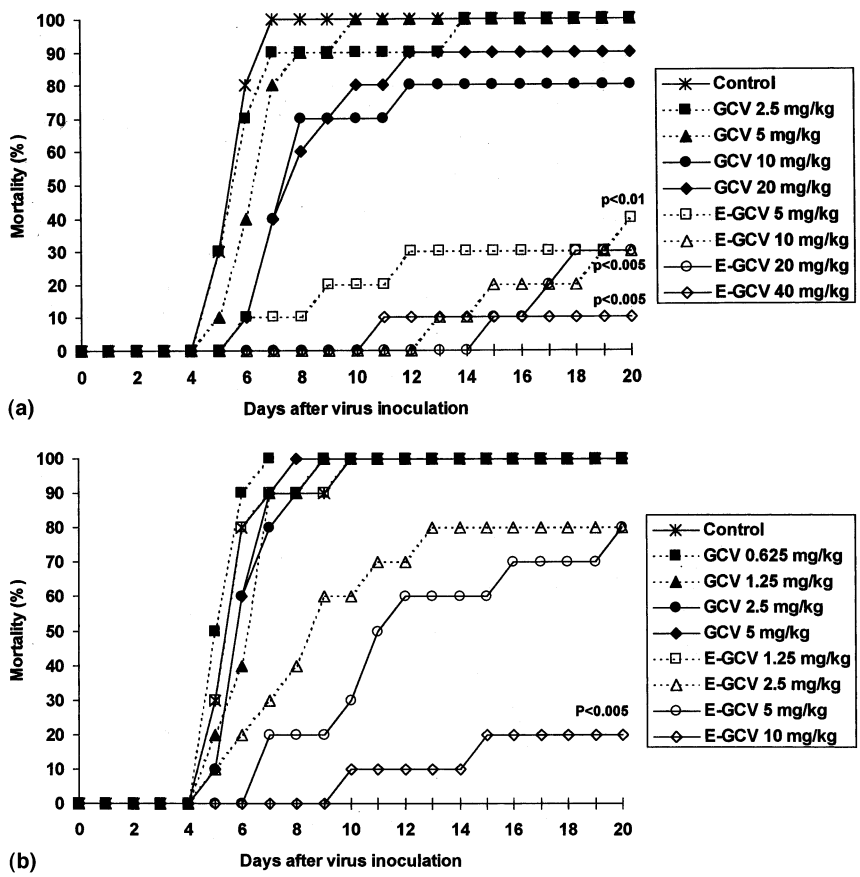

Fig. 3. Intraperitoneal treatment of intracerebral HSV-2 infection in NMRI mice. Animals were treated intraperitoneally with the indicated doses of GCV or E-GCV once daily for 10 days, starting the day of virus infection. Ten mice per group were used. Statistical significance of survival compared with placebo control is indicated only when differences were significant ($P < 0.05$).

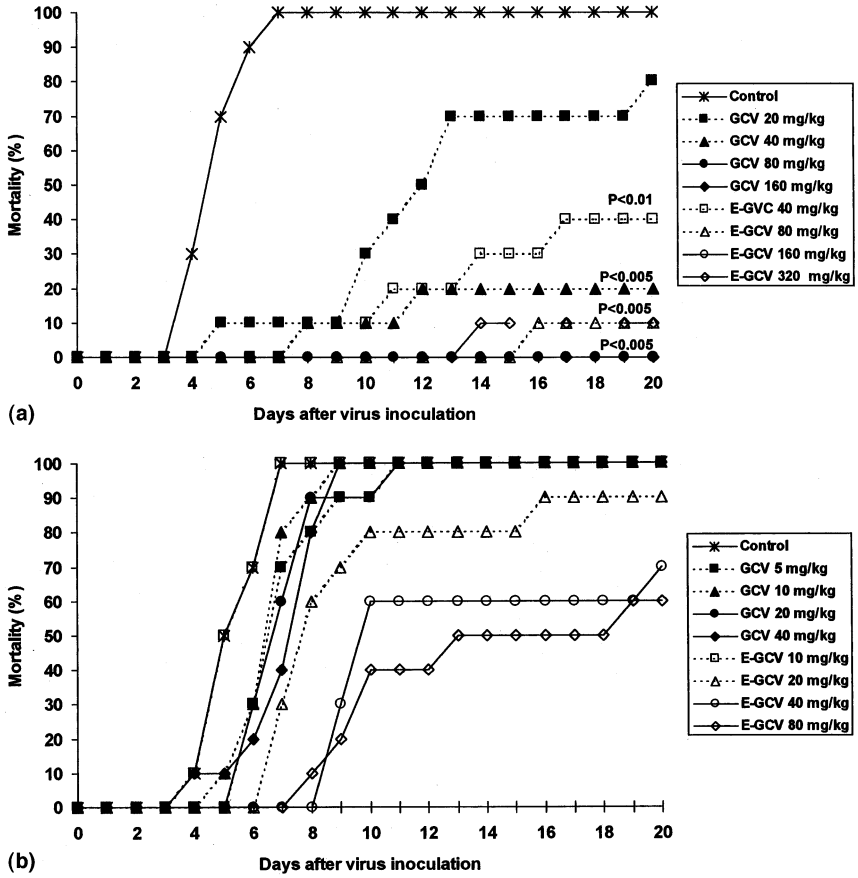

Fig. 4. Peroral treatment of intracerebral HSV-2 infection in NMRI mice. Animals were treated perorally with the indicated doses of GCV or E-GCV once daily for 10 days, starting the day of virus infection. Ten mice per group were used. Statistical significance of survival compared with placebo control is indicated only when differences were significant ($P < 0.05$).

B). GCV given orally at 5, 10, 20 or 40 mg/kg did not affect the mortality rate of mice inoculated intracerebrally with HSV-2. In contrast, E-GCV administered orally at doses of 40 and 80 mg/kg reduced the mortality to 60–70%, with a mean time of death of 11 ($P < 0.05$) and 11.5 days, respectively, as compared to 5.7 days for the control group.

4. Discussion

In the present study, we have demonstrated that the spectrum of antiviral activity of E-GCV is comparable to that of GCV. Similar patterns of sensitivity/resistance to GCV and to E-GCV were observed when different HSV mutants, i.e. ACV^r and/or PFA^r strains, were evaluated. Interestingly, E-GCV proved more inhibitory than GCV to HSV-1 and HSV-2 wild-type and PFA^r strains. Both GCV and E-GCV were greater than 1000-fold less active against TK^- HSV strains, which points to the dependence of the activation of GCV and E-GCV by the viral TK. Although E-GCV proved more active than GCV against HSV-1 and HSV-2 replication in vitro, E-GCV did not prove superior to GCV in their activity against VZV and HCMV.

Not only proved E-GCV more active than GCV against HSV replication in vitro, but it also was much more efficacious than GCV in vivo. When E-GCV was compared to GCV at equimo-

lar doses in the intraperitoneal and peroral treatment of intracerebral HSV-2 infection in NMRI mice, E-GCV was clearly superior to GCV in reducing the mortality rate and mean time of death. Intraperitoneal treatment of HSV-2-infected mice with E-GCV at doses of 10, 20 and 40 mg/kg resulted in a significant decrease in the mortality rate, while GCV at equimolar doses did not affect the mortality rate. Similarly, E-GCV at doses of 20 and 40 mg/kg given orally to mice during 10 days was more effective than the corresponding equimolar doses of GCV. It should be mentioned that even at infrequent dosing (the drugs were administered only once per day) in this severe model, E-GCV proved superior to GCV.

The general mechanism for HCMV, VZV and HSV inhibition by GCV is similar to that observed for inhibition of HSV by ACV (Germershausen et al., 1983; Mar et al., 1985; Smee et al., 1985). Thus, ACV is phosphorylated to the monophosphate form by the viral TK, and cellular enzymes further convert ACV monophosphate to ACV triphosphate, which then inhibits the viral DNA polymerase. Although ACV is phosphorylated less efficiently than GCV (Biron et al., 1985; Smee et al., 1985), ACV triphosphate is considerably more potent than GCV triphosphate in inhibiting the HSV DNA polymerase, and, moreover, ACV is an obligatory chain terminator if incorporated into DNA (Mar et al., 1985). Thus, the efficacy of ACV against HSV can be accounted for by its inhibitory effect on the viral DNA polymerase. In the case of GCV, the selective inhibition of the viral DNA polymerase is enhanced by its efficient intracellular metabolism resulting in the persistence of elevated levels of GCV-triphosphate in infected cells (Biron et al., 1985). Levels of GCV triphosphate are greater than ten-fold higher than levels of ACV triphosphate under similar conditions. Levels of GCV-triphosphate continue to increase slightly and to persist in HSV- or HCMV-infected cells long after the drug has been removed from the culture medium (Biron et al., 1985; Smee et al., 1985).

Balzarini et al. (1998) reported no major differences in the intracellular metabolism of GCV and E-GCV in cell cultures. Both GCV and E-GCV were converted to the mono-, di- and tri-phosphate derivatives of GCV to a markedly higher extent in HSV-1 TK-gene-transfected tumor cells than in the non-transfected tumor cells. However, the mono-, di- and triphosphate metabolites of GCV were retained for a considerably longer time in E-GCV-treated cells than in GCV-treated cells (Balzarini et al., 1998). The longer retention time of the GCV metabolites in E-GCV-treated cells may account for the superior antiviral activity against HSV-1 and HSV-2. However, it remains to be elucidated why E-GCV did not prove more active than GCV against VZV and HCMV. Metabolic studies with GCV and E-GCV in HSV-1-infected cells, as compared to VZV- and HCMV-infected cells, should be conducted to answer this question.

The acyclovir counterpart of E-GCV, ACV elaidate (P-4010), has been evaluated in the female guinea pig model of genital herpes by Jennings et al. (1999). Their results indicated that oral gavage or intraperitoneal injection of different formulations of ACV elaidate had greater efficiency than either ACV or penciclovir (PCV) in reducing the clinical symptoms in this model.

GCV is currently used in the clinic to treat and prevent HCMV disease. Although it has marked antiviral activity against HSV in vitro and in vivo, GCV is not routinely used for the treatment of HSV infections. Yet, several reports have demonstrated the inhibitory effects of GCV on the growth of tumors in mice or rats inoculated in the HSV-1 TK gene-producing packaging cells (Borelli et al., 1988; Culver et al., 1992). In fact, GCV is currently envisaged in the combined gene therapy/chemotherapy of tumors transfected with the HSV-1 TK gene (Raffel et al., 1994; Kun et al., 1995). On the other hand, E-GCV proved markedly more cytostatic to HSV-1 or HSV-2 TK gene-transfected cells than GCV (Balzarini et al., 1998).

The present results, as well as those reported by Balzarini et al. (1998) and Jennings et al. (1999) suggest that E-GCV should be further pursued as a therapeutic modality in the treatment of acute (primary or recurrent) HSV-1 and HSV-2 infections, as well as the chemotherapy of HSV TK gene-transfected tumors.

Acknowledgements

We thank Anita Camps and Willy Zeegers for excellent technical assistance, and Christiane Callebaut, Inge Aerts and Dominique Brabants for dedicated editorial help. This work was supported by Project 3.0026.91 from the Fonds voor Geneeskundig Wetenschappelijk Onderzoek and Project 95/5 from the Belgian Geconcerteerde Onderzoeksacties.

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