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Antiviral activities of nucleosides and nucleotides against wild-type and drug-resistant strains of murine cytomegalovirus

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Abstract

Resistance of human cytomegalovirus to approved antiviral drugs is becoming a problem of increasing concern. In order to further study drug resistance in a related virus, strains of murine cytomegalovirus (MCMV) have been prepared in vitro by extensive adaptation of the virus to increasingly higher concentrations of either ganciclovir, foscarnet, or (*S*)-9-(3-hydroxy-2-[phosphonylmethoxy]propyl)cytosine (HPMPC). Plaque reduction 50% effective concentrations (EC_{50}) for the above inhibitors increased 9-, 7-, and 23-fold, respectively (against the corresponding virus), compared to wild-type MCMV. Each virus was then evaluated against other known anti-MCMV agents to determine cross-resistance patterns. These compounds included 3-hydroxy-phosphonylmethoxypropyl derivatives of adenine (HPMPA) and guanine (HPMPG), 2-phosphonylmethoxyethyl derivatives of adenine (PMEA) and 2,6-diaminopurine (PMEDAP), cyclobutylguanine, acyclovir, and the methylene phosphonate derivatives of acyclovir (SR3722) and ganciclovir (SR3773). The ganciclovir-resistant MCMV was cross-resistant to foscarnet, HPMPA, HPMPC, HPMPG, SR3722, and SR3773. The foscarnet-resistant virus was also resistant to acyclovir, PMEA, PMEDAP, SR3722, and SR3773. The HPMPC-resistant MCMV was cross-resistant to HPMPA, HPMPG, and SR3773. Changes in susceptibility were from 3- to 22-fold relative to the wild-type virus. Virus yield reduction data correlated with the plaque assay results. Only cyclobutylguanine was approximately equally active against wild-type and the three drug-re-

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sistant MCMVs. The patterns of cross-resistance correlated with resistance seen in human cytomegalovirus strains expressing altered DNA polymerase function. The GCV-resistant and HPMPC-resistant viruses were markedly attenuated in their ability to kill severe combined immunodeficient mice.

Keywords: Murine cytomegalovirus; Ganciclovir; Foscarnet; HPMPC; Antiviral; Drug resistance; Nucleoside; Nucleotide

1. Introduction

Two antiviral agents, ganciclovir (Laskin et al., 1987) and foscarnet (Walmsley et al., 1988) are the only approved drugs for the treatment of HCMV infections. Viruses resistant to these drugs have been isolated in laboratory (Biron et al., 1986; Sullivan and Coen, 1991) and clinical (Drew et al., 1991; Erice et al., 1989; Jacobson et al., 1991; Tokumoto and Hollander, 1993) settings. Drug resistance in patients leads to progression of HCMV disease. Ganciclovir resistance may occur by a mutation resulting in a reduced ability of the UL97 viral-encoded enzyme to phosphorylate the drug in infected cells (Littler et al., 1992; Sullivan et al., 1992). Phosphorylation is essential for the antiviral activity of ganciclovir, since the triphosphorylated form is the active antiviral substance (Duke et al., 1986). Mutations in the HCMV DNA polymerase may also confer resistance to ganciclovir (Lurain et al., 1992; Sullivan et al., 1993; Tatarowicz et al., 1992). HCMV resistance to foscarnet is also attributed to mutations in the viral DNA polymerase (Sullivan and Coen, 1991). It has been found that DNA polymerase mutants of HCMV are cross-resistant to experimental anti-HCMV agents which may eventually be approved for clinical use (Lurain et al., 1992; Sullivan and Coen, 1991; Sullivan et al., 1993).

Murine cytomegalovirus (MCMV) is an animal counterpart to HCMV that causes disease in susceptible strains of mice (Kern, 1991). We were interested in determining whether drug-resistant MCMV strains could be prepared in the laboratory for study. Such strains would be useful for determining modes of drug resistance, cross-resistance patterns, and for their utility in animal infection experiments. Correlation with results obtained from HCMV studies could then be made. This report describes the preparation and evaluation of three MCMV strains resistant to ganciclovir, foscarnet, and the experimental agent (*S*)-1-[3-hydroxy-2-(phosphonylmethoxy)propyl]cytosine (HPMPC) that is in clinical development as an anti-HCMV agent (Drew et al., 1994). Compounds evaluated in cross-resistance comparisons included other known active anti-cytomegalovirus agents, such as acyclovir (Burns et al., 1982), cyclobutylguanine (Field et al., 1990) and various nucleotide analogs possessing phosphonate moieties (De Clercq et al., 1987; Duke et al., 1986; Smee et al., 1994).

2. Materials and methods

2.1. Compounds

Acyclovir (ACV, Burroughs Wellcome Co., Research Triangle Park, NC) and ganciclovir (GCV, Syntex Corp., Palo Alto, CA) were purchased from a local pharmacy.

Foscarnet (PFA, Astra Pharmaceutical Products, Westborough, MA) was obtained from Sigma Chemical Company, St. Louis, MO. The 3-hydroxyphosphonylmethoxypropyl derivatives of adenine (HPMPA) and guanine (HPMPG), and the 2-phosphonylmethoxyethyl derivatives of adenine (PMEA) and 2,6-diaminopurine (PMEDAP) were synthesized at the Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague, Czech Republic. Cyclobutylguanine (CBG) was a gift from Bristol-Myers Squibb, Wallingford, CT. The methylene phosphonate derivatives of acyclovir (SR3722) and ganciclovir (SR3773) were prepared at SRI International, Menlo Park, CA. The compounds were prepared as 4 mM stock solutions in cell culture medium for antiviral evaluations.

2.2. Virus and cells

The Smith strain of murine cytomegalovirus (MCMV) and C127I mouse mammary tumor cells were purchased from the American Type Culture Collection, Rockville, MD. The cells were grown in high glucose Dulbecco's medium (DMEM) containing 10% fetal bovine serum (FBS), 50 μg/ml of gentamicin and 0.1% sodium bicarbonate. Mouse embryo fibroblast (MEF) cells were prepared from 15–17 day old mouse embryos. These cells were grown in Eagle's medium (EMEM) along with the other components above. For virus propagation and assay the serum concentration was reduced to 2%.

2.3. Preparation of drug-resistant viruses

Initially the 50% effective concentrations (EC_{50} values) of GCV, PFA, and HPMPC were determined against wild-type MCMV by plaque assay in C127I cells. The virus was passaged in MEF cells starting at each EC_{50} concentration. The cell cultures were incubated up to a month (with weekly changes in the culture medium) until virus-induced cytopathology was maximal. With each subsequent passage of the virus, the drug was increased by a 2-fold or less increment of concentration. Sometimes with low virus recovery after a passage in the presence of drug, the virus had to be passaged in drug-free medium to increase its titer prior to continuing drug selection pressure. It took 6–9 months to develop the PFA-resistant (PFA-r) and HPMPC-resistant (HPMPC-r) viruses and about 18 months to develop the GCV-resistant (GCV-r) virus. The highest concentrations of PFA, GCV, and HPMPC used to grow the corresponding drug-resistant viruses were 1000, 200, and 20 μM, respectively. The final stock of each strain was prepared in drug-free medium. Antiviral assays were performed using viruses which had not been plaque purified. Subsequently, plaque purified stocks have been prepared which may allow for characterization of the drug-resistant genotype.

2.4. Plaque reduction assays

The antiviral activities of the test compounds were determined using confluent monolayers of C127I cells in 12-well plates infected with the different strains of MCMV, using about 100 virus plaque forming units (PFU)/well. After 1 h of virus

adsorption, the medium was replaced with test compounds in the above DMEM medium which also contained 0.5% SeaPlaque agarose (FMC Corp., Rockland, ME). After 7 (wild-type virus) to 10 days (drug-resistant viruses, which grow slowly) the wells were overlayed with 1 ml of 10% buffered formalin for 15 min. Then the agar and formalin were removed and the monolayers were stained with 0.1% crystal violet in 20% ethanol for 5 min. After rinsing off excess stain, the plaques were counted with the aid of a Plaque Viewer (Bellco Labs, Vineland, NJ). Fifty percent effective concentration (EC_{50}) values were estimated by plotting on semi-log paper.

2.5. Virus yield reduction assays

Virus (about 300 PFU/well) in 24-well plates of C127I cells was grown in the presence of drug for 7 to 10 days until virus-induced cytopathology was maximal in drug-free wells. Two wells were used per dilution of compound. Then the cells and supernate from each set of wells were collected and sonicated 1 min. Samples were stored frozen at −80°C until titrated for virus. Titrations were conducted in 96-well plates of C127I cells by end point dilution method (Reed and Muench, 1938). Each sample was titrated in quadruplicate with a ProPette (Perkin Elmer Cetus, Emeryville, CA), using a 4-fold dilution scheme through 12 dilutions. Virus titers are expressed as $\log_{10}$ cell culture infectious doses ($CCID_{50}$) per ml.

2.6. Infection of mice with drug-resistant viruses

In order to test the virulence of the drug-resistant MCMVs and to explore the suitability of the viruses for animal chemotherapy experiments, each virus was inoculated into groups of 15 severe combined immunodeficient (SCID) mice at 3 to 5×10^5 PFU/animal. These animals are highly susceptible to wild-type MCMV infection (Smee et al., 1992). Deaths were recorded daily through 110 days. Other mice were inoculated in parallel in order to obtain tissues near the time of death for subsequent virus assays. The recovered viruses were titrated for drug susceptibility to GCV, PFA, and HPMPC to determine if passage in the mice caused reversion to the wild-type phenotype.

3. Results

3.1. Antiviral activities in cell culture

The comparative effects of eleven compounds against wild-type, GCV-r, PFA-r, and HPMPC-r strains of MCMV are presented in Table 1. GCV exhibited a 9.5-fold decrease in sensitivity toward the GCV-r virus compared to wild-type MCMV. Similar decreases in antiviral sensitivity of this virus to PFA, HPMPA, HPMPC, and SR3773 were apparent. Only slight changes in EC_{50} values of HPMPG and SR3722 were evident. PFA showed a 7.1-fold decrease in activity toward the PFA-r virus compared to wild-type MCMV. Compounds which also showed less potency toward the PFA-r virus included ACV, PMEA, PMEDAP, SR3722 and SR3773. HPMPC was found to be

Table 1
Activities of compounds against wild-type and drug-resistant strains of MCMV, determined by plaque reduction assays in C127I cells

Compound	50% Effective concentration (EC_{50}, μM) [a]			
	Wild-type MCMV	GCV-r MCMV	PFA-r MCMV	HPMPC-r MCMV
PFA	90 ± 4.8	650 ± 165 (7.2) [b]	640 ± 72 (7.1)	37 ± 19 (−2.4)
GCV	6.0 ± 1.1	57 ± 5.8 (9.5)	6.3 ± 0.8	11.0 ± 4.1
ACV	4.0 ± 0.8	5.9 ± 0.8	20.3 ± 2.5 (5.1)	2.7 ± 0.8
CBG	2.6 ± 0.2	2.6 ± 0.1	3.1 ± 0.3	2.1 ± 0.6
PMEA	2.3 ± 0.1	2.4 ± 0.9	11.1 ± 1.4 (4.8)	2.3 ± 0.9
PMEDAP	0.3 ± 0.1	0.3 ± 0.1	0.9 ± 0.2 (3.0)	0.3 ± 0.1
HPMPA	0.9 ± 0.2	8.5 ± 1.1 (9.4)	0.6 ± 0.1	11.0 ± 4.4 (12.2)
HPMPC	0.2 ± 0.1	1.8 ± 0.2 (9.0)	0.3 ± 0.1	4.7 ± 2.1 (23.5)
HPMPG	0.8 ± 0.3	2.6 ± 0.5 (3.3)	0.8 ± 0.1	3.1 ± 0.8 (3.9)
SR3722	4.3 ± 1.2	11.2 ± 3.3 (2.6)	24.3 ± 3.2 (5.7)	3.1 ± 0.8
SR3773	0.6 ± 0.1	5.2 ± 0.6 (8.7)	1.9 ± 0.4 (3.0)	3.7 ± 1.9 (6.2)

[a] Mean of 3 to 4 determinations ± S.D.
[b] Fold change in sensitivity compared to wild-type virus (only values greater than 2-fold are indicated). A negative value indicates greater sensitivity toward the drug-resistant virus.

23.5-fold less effective against the HPMPC-r MCMV than the wild-type virus. Other compound exhibiting less potency toward the HPMPC-r virus included HPMPA, HPMPG, and SR3773. Of interest was the fact that PFA was more inhibitory to the HPMPC-r virus than to the wild-type MCMV.

A considerable degree of cross-resistance among GCV and HPMP compounds occurred with the GCV-r and HPMPC-r viruses. The PFA-r virus was not cross-resistant to GCV and HPMP compounds, but was to ACV and the ACV analog SR3722. Overall, CBG and PMEDAP were the least affected by drug resistance in terms of decreases in antiviral potency, whereas SR3773 was the only compound that became less potent against all three of the drug-resistant viruses.

The activities of six of the compounds were compared against drug-resistant and wild-type viruses in virus yield reduction assays (Fig. 1), the results generally correlating with the plaque reduction data presented in Table 1. PFA was more effective against HPMPC-r virus but less active against GCV-r and PFA-r viruses than wild-type MCMV. GCV was less active against the GCV-r virus than the other viruses. CBG exhibited the same degree of virus-inhibitory activity against all four of the viruses. HPMPC was less active against the GCV-r and HPMPC-r strains of virus. The GCV-r and PFA-r viruses were less affected by SR3722 than were the other viruses. All three drug-resistant viruses were less sensitive to SR3773 than was the wild-type MCMV.

3.2. Animal experiments

The virulence of the drug-resistant and wild-type MCMVs was determined in SCID mice (Table 2). Animals inoculated with wild-type virus died in about 12 days. The PFA-r virus showed a reasonable degree of virulence, with all mice dying at about 20

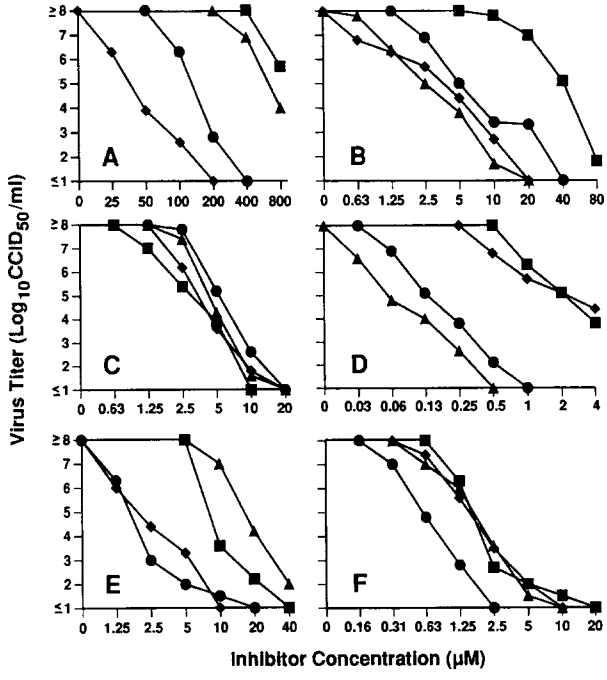

Fig. 1. Effects of compounds on wild-type and drug-resistant MCMV yields from C127I cells. Substances evaluated include PFA (A), GCV (B), CBG (C), HPMPC (D), SR3722 (E) and SR3773 (F). Symbols: ●, wild-type virus; ■, GCV-r virus; ▲, PFA-r virus; ◆, HPMPC-r virus.

days. The GCV-r virus was much less virulent, with mice dying between 60 and 80 days. The HPMPC-r virus was almost completely attenuated since only one death occurred at 69 days. Viruses recovered from the mice at or near death still exhibited drug-resistant phenotypes against the respective compound used to induce the drug resistance.

Table 2
Effects of wild-type and drug-resistant strains of MCMV on survival of SCID mice

MCMV strain [a]	Dead/total (%)	Mean day to death [b]
Wild-Type	15/15 (100)	12.5 ± 1.8 [c]
GCV-r	14/15 (93)	69.6 ± 12.8
PFA-r	15/15 (100)	20.0 ± 2.5
HPMPC-r	1/15 (7)	69.0 ± 0.0

[a] Animals were inoculated i.p. with 3 to 5×10^5 PFU of virus.
[b] Survivors living 110 days were not included in these data.
[c] Standard deviation.

4. Discussion

The patterns of cross-resistance reported here for MCMV correlate with similar studies of HCMV, implicating the viral DNA polymerase as the site of mutation. An HCMV isolate resistant to PFA was reported to be cross-resistant to phosphonoacetic acid (PAA) and acyclovir, but not to HPMPC, HPMPA, or ganciclovir (Sullivan and Coen, 1991). Certain HCMV isolates resistant to GCV were cross-resistant to PFA and/or to HPMPC (Lurain et al., 1992; Tatarowicz et al., 1992; Sullivan et al., 1993). CBG was nearly equally active against GCV-resistant and GCV-sensitive strains of HCMV (Clement and Kern, 1991). These were precisely the types of patterns reported here in MCMV studies. Previously, Burns et al. (1982) reported a strain of MCMV that was resistant to both acyclovir and PAA. In addition to being resistant to ACV, our PFA-resistant virus was also resistant to PMEA, PMEDAP, SR3722, and SR3773. Foster et al. (1991) reported that a herpes simplex virus type 1 that was resistant to PMEA was also resistant to PFA. Since PMEA and PMEDAP are closely related in structure, it is logical that resistance to one would confer resistance to the other. Similarly, cross-resistance of this virus to ACV and ACV phosphonate (SR3722) may be due to structural similarity. This argument cannot be applied to the GCV-r strain, which is resistant to SR3722 but not to ACV.

SR3773 exhibited a degree of virus-inhibitory activity against the resistant strains different from that of GCV (i.e., the PFA-r and HPMPC-r viruses were resistant to SR3773, but not to GCV). Since all three drug-resistant viruses were resistant to SR3773, this compound may be a poor candidate drug in patients who require alternative therapy due to emergence of drug resistant virus. Overall, CBG was the most effective compound since it was equally active against all of the MCMVs tested. This may have positive implications in the treatment of HCMV infections. To date we have been unable to create a strain of MCMV that is resistant to this compound, but efforts are continuing.

We observed that virus plaques from drug-resistant viruses took about three days longer than wild-type virus to reach the same relative size (10 versus 7 days). These results imply that the drug-resistant viruses grew more slowly in culture, which means either that the overall yield from a single replicative cycle is less with the resistant viruses or else that the time to complete a cycle of replication is longer than with wild-type virus. It will require further research to clarify this issue.

Attenuation of virulence naturally arises when wild-type virus is grown in cell culture and is subsequently used in mice (Selgrade et al., 1981). Since numerous cell culture passages were required to prepare the drug-resistant viruses, attenuation was expected. Attenuation of virulence is largely reversed, however, if wild-type cell culture passaged virus is inoculated into immunosuppressed mice (Selgrade et al., 1981). In our studies, infection of immunosuppressed mice with the cell culture passaged drug-resistant viruses yielded mixed results in terms of virulence. In mice the PFA-r virus was reasonably virulent whereas the GCV-r and HPMPC-r viruses were more attenuated. It is our hypothesis that additional attenuation arises as a result of certain mutations in the viral DNA polymerase, as was suggested by the differential effects of the three drug-resistant viruses in mice. The PFA-r virus must not be too much different than

wild-type virus since it exhibited comparable virulence in vivo. For each of these drug-resistant viruses, one passage through the mice did not alter the drug-resistance phenotype with regard to the original compound used to induce the resistance in cell culture. The effects of further passages of these viruses in vivo on drug susceptibility have not been examined, however.

We are not certain to what extent drug resistance mutations contribute to attenuation in vivo. It may be possible to increase the virulence of the GCV-r and HPMPC-r strains by multiple adaptive passages of these viruses in mice. Such studies in our laboratory have been hindered due to the low virus recovery from mice, necessitating passage in cell culture to boost virus titers before new animal infections can be performed. Thus, any increase in virulence in vivo may be lost again during cell culturing prior to infecting a new group of mice. At this stage of development, the PFA-r and GCV-r strains of MCMV may be useful for studying the activities of antiviral agents in mice, whereas the HPMPC-r virus may be limited to cell culture experiments only.

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References

Biron, K.K, Fyfe, J.A., Stanat, S.C., Leslie, L.K., Sorrell, J.B., Lambe, C.U. and Coen, D.M. (1986) A human cytomegalovirus mutant resistant to the nucleoside analog 9-{[2-hydroxy-1-(hydroxymethyl)ethoxy]-methyl}guanine (BW B759U) induces reduced levels of BW B759U triphosphate. Proc. Natl. Acad. Sci. USA 83, 8769–8773.

Burns, W.H., Wingard, J.R., Sandford, G.R., Bender, W.J. and Saral, R. (1982) Acyclovir in mouse cytomegalovirus infections. Am. J. Med. 73, 118–124.

Clement, J.J. and Kern, E.R. (1991) Cyclobutyl compounds as antiviral agents. Transplant Proc. 23 (Suppl. 3), 159–161.

De Clercq, E., Sakuma, T., Baba, M., Pauwels, R., Balzarini, J., Rosenberg, I. and Holy, A. (1987) Antiviral activity of phosphonylmethoxyalkyl derivatives of purine and pyrimidines. Antiviral Res. 8, 261–272.

Drew, W.L., Lalezari, J.P., Glutzer, E., Martin, J.C., Fisher, P.E. and Jaffe, H.S. (1994) The safety, pharmacokinetics, and anti-CMV activity of weekly HPMPC in HIV positive patients excreting CMV. Antiviral Res. 23 (Suppl. 1), 94.

Drew, W.L., Miner, R.C., Busch, D.F., Follansbee, S.E., Gullet, J., Mehalko, S.G., Gordon, S.M., Owen Jr., W.F., Matthews, T.R., Buhles, W.C. and DeArmond, B. (1991) Prevalence of resistance in patients receiving ganciclovir for serious cytomegalovirus infection. J. Infect. Dis. 163, 716–719.

Duke, A.E., Smee, D.F., Chernow, M., Boehme, R. and Matthews, T.R. (1986) In vitro and in vivo activities of phosphate derivatives of 9-(1,3-dihydroxy-2-propoxymethyl)guanine against cytomegaloviruses. Antiviral Res. 6, 299–308.

Erice, A., Chou, S., Biron, K.K., Stanat, S.C., Balfour, H.H. and Jordan, M.C. (1989) Progressive disease due to ganciclovir-resistant cytomegalovirus in immunocompromised patients. New Engl. J. Med. 320, 289–292.

Field, A.K., Tuomari, A.V., McGeever-Rubin, B., Terry, B.J., Mazina, K.E., Haffey, M.L., Hagen, M.E., Clark, J.M., Braitman, A., Slusarchyk, W.A., Young, M.G. and Zahler, R. (1990) (+)-(1a,2b,2a)-9-[2,3-

bis(hydroxymethyl)cyclobutyl]guanine [(+)-BHCG or SQ 33054]: a potent and selective inhibitor of herpesviruses. Antiviral Res. 13, 41–52

Foster, S.A., Cerny, J. and Cheng, Y.-C. (1991) Herpes simplex virus-specified DNA polymerase is the target for the antiviral action of 9-(2-phosphonylmethoxyethyl)adenine. J. Biol. Chem. 266, 238–244.

Jacobson, M.A., Drew, W.L., Feinberg, J., O'Donnell, J.J., Whitmore P.V., Miner, R.D. and Pareti, D. (1991) Foscarnet therapy for ganciclovir-resistant cytomegalovirus retinitis in patients with AIDS. J. Infect. Dis. 163, 1348–1351.

Laskin, O.L., Cederberg, D.M., Mills, J., Eron, L.J., Mildvan, D. and Spector, S.S. (1987) Ganciclovir for the treatment and suppression of serious infections caused by cytomegaloviruses. Am. J. Med. 83, 201–207.

Littler, E., Stuart, A.D. and Chee, M.S. (1992) Human cytomegalovirus UL97 open reading frame encodes a protein that phosphorylates the antiviral nucleoside analogue ganciclovir. Nature 358, 160–162.

Lurain, N.S., Thompson, K.D., Holmes, E.W. and Read, G.S. (1992) Point mutations in the DNA polymerase gene of human cytomegalovirus that result in resistance to antiviral agents. J. Virol. 66, 7146–7152.

Kern, E.R. (1991) Value of animal models to evaluate agents with potential activity against human cytomegalovirus. Transplant. Proc. 23 (Suppl. 3), 152–155.

Reed, L.J. and Muench, M. (1938) A simple method of estimating fifty percent end points. Am. J. Hyg. 27, 493–498.

Selgrade, M.K., Nedrud, J.G., Collier, A.M. and Gardner, D.E. (1981) Effects of cell source, mouse strain, and immunosuppressive treatment on production of virulent and attenuated murine cytomegalovirus. Infect. Immun. 33, 840–847.

Smee, D.F., Morris, J.L.B., Leonhardt, J.A., Mead, J.R., Holy, A. and Sidwell, R.W. (1992) Treatment of murine cytomegalovirus infections in severe combined immunodeficient mice with ganciclovir, (S)-1-[3-hydroxy-2-(phosphonylmethoxy)propyl]cytosine (HPMPC), interferon, and bropirimine. Antimicrob. Agents Chemother. 36, 1837–1842.

Smee, D.F., Sugiyama, S.T. and Reist, E.J. (1994) Nucleotide analogs related to acyclovir and ganciclovir are effective against murine cytomegalovirus infections in BALB/c and severe combined immunodeficient mice. Antimicrob. Agents Chemother. 38, 2165–2168.

Sullivan, V. and Coen, D.M. (1991) Isolation of foscarnet-resistant human cytomegalovirus patterns of resistance and sensitivity to other antiviral drugs. J. Infect. Dis. 164, 781–784.

Sullivan, V., Biron, K.K., Talarico, C., Stanat, S.C., Davis, M., Pozzi, L.M. and Coen, D.M. (1993) A point mutation in the human cytomegalovirus DNA polymerase gene confers resistance to ganciclovir and phosphonylmethoxyalkyl derivatives. Antimicrob. Agents Chemother. 37, 19–25.

Sullivan, V., Talarico, C.L., Stanat, S.C., Davis, M., Coen, D.M. and Biron, K.K. (1992) A protein kinase homologue controls phosphorylation of ganciclovir in human cytomegalovirus-infected cells. Nature 358, 162–164.

Tatarowicz, W.A., Lurain, N.S. and Thompson, K.D. (1992) A ganciclovir-resistant clinical isolate of human cytomegalovirus exhibiting cross-resistance to other DNA polymerase inhibitors. J. Infect. Dis. 166, 904–907.

Tokumoto, J.I.N. and Hollander, H. (1993) Cytomegalovirus polyradiculopathy caused by a ganciclovir-resistant strain. Clin. Infect. Dis. 17, 854–856.

Walmsley, S.L., Chew, E., Fanning, M.M., Read, S.E., Vellend, H., Salit, I. and Rachlis, A. (1988) Treatment of cytomegalovirus retinitis with trisodium phosphonoformate hexahydrate (foscarnet). J. Infect. Dis. 157, 569–572.