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Antiviral activity of 9-[[(ethoxyhydroxyphosphinyl)-methoxy]methoxy]guanine against cytomegalovirus and herpes simplex virus

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Abstract

An isosteric analog of 9-[2-(phosphonomethoxy)ethyl]guanine (PMEG), 9-[[(ethoxyhydroxyphosphinyl)methoxy]methoxy]guanine (SKI 1008), was evaluated for its in vitro antiviral activity against herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2), murine cytomegalovirus (MCMV), and human cytomegalovirus (HCMV), and its in vivo antiviral efficacy against MCMV in mice. The in vitro anti-HSV activity of SKI 1008 was much lower than that of acyclovir, even though SKI 1008 showed similar antiviral activity against thymidine kinase positive (TK^+) and thymidine kinase negative (TK^-) strains. Like ganciclovir and PMEG, SKI 1008 selectively inhibited plaque formation of MCMV; the 50% effective concentration (EC_{50}) and the 50% cytotoxic concentration (CC_{50}) of SKI 1008, ganciclovir, and PMEG being 0.51 and 600, 1.65 and 461, and 0.06 and 12.1 μg/ml, respectively. The in vitro EC_{50} value of SKI 1008 against HCMV was comparable to that of ganciclovir (0.24 vs 0.16 μg/ml) and was 12-fold higher than that of PMEG in a plaque reduction assay, but the therapeutic indices (the ratios of CC_{50} to EC_{50}) of SKI 1008 and ganciclovir were higher than that of PMEG. The in vivo antiviral efficacy of SKI 1008 in MCMV-infected normal BALB/c and severe combined immunodeficiency (SCID) mice was lower than that of ganciclovir in terms of mortality and mean survival time.

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1. Introduction

Human cytomegalovirus (HCMV) is a herpesvirus which causes serious infections in immunologically immature or compromised hosts such as neonates, organ transplant recipients, cancer patients, and AIDS patients (Alford and Britt, 1990). Effective therapy for HCMV-infected individuals is limited due to the toxic side effects of currently available therapeutic agents such as 9-(1,3-dihydroxy-2-propoxymethyl)guanine (DHPG, ganciclovir) and the trisodium salt of phosphonoformic acid (PFA, foscarnet), and the need to maintain continuous therapy to prevent recurrence (Koretz et al., 1986; Balfour, 1990; Drew et al., 1991; Meyers, 1991). Thus, the search for more effective and less toxic antiviral drugs for the chemotherapy of HCMV infections is of high priority.

Recently, metabolically and chemically stable acyclic nucleoside phosphonate analogs, (*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine (HPMPC) (De Clercq et al., 1987; Snoeck et al., 1988; Andrei et al., 1991; Neyts et al., 1992) and 9-[2-(phosphono-methoxy)ethyl]guanine (PMEG) (Reist et al., 1988; Sidwell et al., 1988; Kim et al., 1991) have been reported as potent and selective inhibitors against various DNA virus infections including HCMV. It has also been reported that the monoethyl esters of acyclic nucleoside phosphonates displayed comparable or even better activity against CMV than the corresponding diacid (Reist et al., 1989).

We have recently described an isostere of PMEG monoethyl ester, 9-[[(ethoxyhy-droxyphosphinyl)methoxy]methoxy]guanine (SKI 1008, Fig. 1), which showed in vitro anti-HCMV activity comparable to that of ganciclovir (Kim et al., 1994). In this study, we have further examined the in vitro antiviral activity of SKI 1008 against CMV and herpes simplex virus (HSV) and investigated the antiviral efficacy of SKI 1008 in MCMV-infected normal BALB/c and severe combined immunodeficiency (SCID) mice.

2. Materials and methods

2.1. Compounds

SKI 1008 was synthesized at the Life Science Research Center of Sunkyong Industries (Suwon-Si, Korea) according to the method previously described (Kim et al., 1994). PMEG was synthesized according to the method previously described by Bronson et al. (1989). Ganciclovir was purchased from Syntex Inc. (Palo Alto, CA, USA). Acyclovir was obtained from Sigma Chemical Company (St. Louis, MO, USA).

2.2. Mice

Six-week-old male specific pathogen free (SPF) BALB/c mice were obtained from Shizuoka Laboratory Animal Center (SLC) (Hamamatsu, Japan) and 6-week-old male

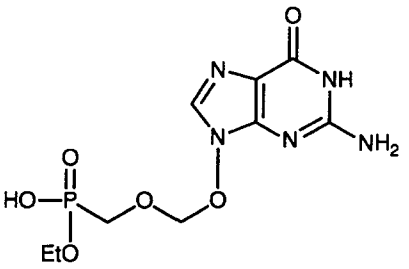

Fig. 1. Chemical structure of 9-[[(ethoxyhydroxyphosphinyl)methoxy]methoxy]guanine (SKI 1008).

severe combined immunodeficiency (SCID) mice were purchased from Japan CLEA Inc. (Tokyo, Japan). All mice were housed under SPF condition during the experiments, and food and water were provided ad libitum.

2.3. Cells

African green monkey kidney (Vero) and human embryonic lung (HEL) fibroblast cells were obtained from American Type Culture Collection (ATCC, MD, USA). They were maintained and passaged in Eagle's minimum essential medium (EMEM) (Gibco, MD, USA) containing 10% fetal bovine serum (FBS) (Gibco, MD, USA). Primary mouse embryo fibroblast (MEF) cells were prepared from BALB/c mouse embryo by mechanical and enzymatic treatment and were used between passage 2 and passage 4.

2.4. Viruses

HSV-1 (KOS strain, thymidine kinase-positive), TK^- HSV-1 (KOS strain, thymidine kinase-negative), HSV-2 (186 strain, thymidine kinase-positive) and TK^- HSV-2 (186 strain, thymidine kinase-negative) were kindly provided by Prof. Y. Nishiyama (Nagoya University, Nagoya, Japan). Mouse CMV (Smith strain) and human CMV (AD169 strain) were obtained from ATCC. Stocks of HSV and HCMV strains were prepared in Vero cells and HEL cells, respectively, and were stored at −70°C. Mouse CMV was maintained by serial passage in BALB/c mice. In brief, 5-week-old mice were inoculated i.v. with 1×10^5 plaque forming units (PFU) of MCMV, and salivary glands were collected at day 10 postinfection. Collected salivary glands were homogenized to be 10% homogenates in EMEM supplemented with 10% FBS. Virus in salivary glands was titrated on MEF cells and determined to be about 4×10^7 PFU/ml.

2.5. Plaque reduction assay

Confluent cells in 24-well multidish plates were infected with about 60 PFU of each virus in culture medium per well. After a 1-h adsorption period at 37°C in an incubator containing 5% CO_2, the medium containing residual virus was removed, and 1 ml of EMEM containing 2% FBS, 1% methyl cellulose, and various concentrations of test compounds was added to each well. The assay was run in triplicate. After incubation at

37°C in an incubator containing 5% CO_2 for appropriate periods (HCMV, 10 days; MCMV, 6 days; HSV-1 and HSV-2, 3 days), plaques were fixed with methanol, stained with crystal violet, and counted. The concentration of test compound which reduced plaque number of control by 50%, EC_{50}, was calculated from dose–response curve plotted with probit analysis.

2.6. Cytotoxicity test

Vero, HEL, and MEF cells in culture medium were seeded at 1×10^4 cells per well into 96-well microtiter plates and allowed to proliferate for 24 h. Thereafter, 9 different concentrations of test compounds were added in triplicate. After a 3-day incubation at 37°C in an incubator containing 5% CO_2, the viable cell number of each concentration was determined by the MTT assay (Raju et al., 1989). The concentration of test compound which reduced optical density (O.D.) of control by 50%, CC_{50}, was calculated from dose–response curves plotted with probit analysis.

2.7. In vivo antiviral efficacy test

For in vivo antiviral efficacy test in BALB/c mice, mice were infected i.v. with 4×10^6 PFU of MCMV, which was estimated to be lethal dose $(LD)_{90}$ value in the preliminary mortality study. Each group consisted of 10 mice. Test compounds dissolved in phosphate-buffered saline were administered i.p. according to the two experimental schedules, once-daily for 5 consecutive days and once every 3 days for 15 days, starting at 24 h postinfection. Death and body weight change were recorded for 21 days after virus inoculation. For in vivo antiviral efficacy test in SCID mice, mice were infected i.v. with 8×10^5 PFU of MCMV. Each group consisted of 10 mice. Test compounds dissolved in phosphate-buffered saline were administered i.p. once daily for 5 consecutive days starting at 24 h postinfection. Death and body weight change were recorded for 30 days after virus inoculation. The parameters used to assess in vivo antiviral efficacy included prevention of mortality and delay in mean survival day to death.

2.8. Statistical analysis

The two-tailed Fisher's exact test was used for determination of significant difference in survival rate, and the two-tailed Mann–Whitney *U*-test was used for determination of significant difference in mean survival day. *P*-values of < 0.05 were considered statistically significant.

3. Results

3.1. In vitro anti-HSV activity of SKI 1008 and acyclovir

The in vitro anti-HSV activity and cytotoxicity of SKI 1008 and acyclovir are summarized in Table 1. The antiviral activity of SKI 1008 against TK^+ HSV-1 and

Table 1
Anti-HSV activity of SKI 1008 and acyclovir in Vero cells [a]

Type (strain, phenotype)	EC_{50} (μg/ml) [b]		CC_{50} (μg/ml) [c]		TI [d]	
	SKI 1008	Acyclovir	SKI 1008	Acyclovir	SKI 1008	Acyclovir
HSV-1 (KOS, TK^+)	30.8	1.87	> 1000	340	> 32	182
HSV-1 (KOS, TK^-)	23.0	26.4			> 43	13
HSV-2 (186, TK^+)	83.5	2.62			> 12	130
HSV-2 (186, TK^-)	35.6	> 100			> 28	< 3.4

[a] Values are the mean of at least two independent experiments run in triplicate.
[b] Concentration required to reduce the number of plaques by 50% of the virus-infected control.
[c] Concentration required to reduce the O.D. value by 50% of control.
[d] Therapeutic index (the ratio of CC_{50} to EC_{50}).

HSV-2 was much lower than that of acyclovir; however, the difference of antiviral activity of SKI 1008 between TK^+ and TK^- strains of HSV was not observed. The cytotoxicity of SKI 1008 in Vero cells was lower than that of acyclovir; the CC_{50} values of SKI 1008 and acyclovir were > 1000 and 340 μg/ml, respectively.

3.2. *In vitro anti-CMV activity of SKI 1008, ganciclovir, and PMEG*

The in vitro antiviral activity and cytotoxicity of SKI 1008 against MCMV in MEF and HCMV in HEL was compared with those of ganciclovir and PMEG (Table 2). Compared with ganciclovir and PMEG, SKI 1008 selectively inhibited plaque formation by MCMV; the EC_{50} and CC_{50} values of SKI 1008, ganciclovir, and PMEG were 0.51 and 600, 1.65 and 461, and 0.06 and 12.1 μg/ml, respectively. The in vitro EC_{50} value of SKI 1008 against HCMV was comparable to that of ganciclovir (0.24 vs 0.16 μg/ml) and was 12-fold higher than that of PMEG in a plaque reduction assay, but the therapeutic indices (the ratios of CC_{50} to EC_{50}) of SKI 1008 and ganciclovir were higher than that of PMEG.

Table 2
Antiviral activity of SKI 1008, ganciclovir, and PMEG against MCMV (Smith strain) in MEF cells and HCMV (AD169) in HEL cells [a]

Compound	EC_{50} (μg/ml) [b]		CC_{50} (μg/ml) [c]		TI [d]	
	MCMV	HCMV	MEF	HEL	MCMV/MEF	HCMV/HEL
SKI 1008	0.51	0.24	600	279	1176	1163
Ganciclovir	1.65	0.16	461	449	279	2806
PMEG	0.06	0.02	12.1	7.0	202	350

[a] Values are the mean of at least two independent experiments run in triplicate.
[b] Concentration required to reduce the number of plaques by 50% of the virus-infected control.
[c] Concentration required to reduce the O.D. value by 50% of control.
[d] Therapeutic index (the ratio of CC_{50} to EC_{50}).

Table 3
Effects of SKI 1008 and ganciclovir on MCMV-induced mortality in BALB/c mice [a]

Compound	Expt.	Dose (mg/kg/day)	% Survival	Mean survival [b] (days)
SKI 1008	1	10.0	60	12.3 ± 1.0
	1	2.0	30	12.1 ± 1.7
Ganciclovir	1	10.0	100 [c]	> 21.0
	1	2.0	80	11.5 ± 0.7
SKI 1008	2	20.0	80	11.5 ± 3.5
	2	10.0	60	10.5 ± 1.7
	2	2.0	50	12.4 ± 0.9
Ganciclovir	2	20.0	100 [c]	> 21.0
	2	10.0	80	11.5 ± 2.1
	2	2.0	50	10.4 ± 2.7
Untreated		–	40	12.3 ± 1.4

[a] Ten mice per group were infected i.v. with 4×10^6 PFU of MCMV (Smith strain) and treated i.p. with test compounds according to the two experimental schedules, once daily for 5 consecutive days (Expt. 1) and once every 3 days for 15 days (Expt. 2), starting at 24 h postinfection. Expts. 1 and 2 were run concurrently.
[b] Each value represents the mean ± S.E.M.
[c] Significantly different from untreated group ($P < 0.05$).

3.3. Effect of SKI 1008 and ganciclovir on MCMV-induced mortality in BALB/c mice

The effects of SKI 1008 and ganciclovir on MCMV-induced mortality in BALB/c mice are presented in Table 3. MCMV-infected mice receiving no antiviral therapy developed a wasting syndrome by day 3 postinfection, started to die a few days later, and showed a survival rate of 40%. Following a once daily 5-day consecutive treatment schedule (Expt. 1), the groups treated with either SKI 1008 or ganciclovir at doses of 2.0 or 10.0 mg/kg/day showed survival rates of 30 and 60%, and 80 and 100%, respectively.

On an intermittent treatment schedule (Expt. 2), both SKI 1008 and ganciclovir dose-dependently protected the mortality of MCMV-infected mice. The survival rates of the groups treated with either SKI 1008 or ganciclovir at doses of 2.0, 10.0, or 20.0 mg/kg/injection were 50, 60, and 80%, and 50, 80, and 100%, respectively.

Ganciclovir completely protected MCMV-induced mortality, and mice receiving ganciclovir did not show clinical signs, such as wasting and ruffling, when administered as either once-daily 5-day consecutive treatment of 10.0 mg/kg/day or intermittent treatment of 20.0 mg/kg/injection.

3.4. Effect of SKI 1008 and ganciclovir on MCMV-induced mortality in SCID mice

Table 4 shows the effects of SKI 1008 and ganciclovir on MCMV-induced mortality of SCID mice. Both SKI 1008 and ganciclovir caused an increase in the mean survival day, as compared with that of untreated group ($P < 0.01$), although all the mean

Table 4
Effect of SKI 1008 and ganciclovir on MCMV-induced mortality in SCID mice [a]

Compound	Dose (mg/kg/day)	% Survival	Mean survival [b] (days)
SKI 1008	20.0	0	17.1 ± 1.9 [c]
	10.0	0	17.0 ± 1.8 [c]
Ganciclovir	20.0	0	25.2 ± 1.6 [c,d]
	10.0	0	23.9 ± 2.2 [c,d]
Untreated	–	0	13.7 ± 1.3

[a] Ten mice per group were infected i.v. with 8×10^5 PFU of MCMV (Smith strain) and treated i.p. with test compounds once daily for 5 consecutive days starting at 24 h postinfection.
[b] Each value represents the mean ± S.E.M.
[c] Significantly different from untreated group ($P < 0.01$).
[d] Significantly different from SKI 1008-treated groups ($P < 0.01$).

survival days for the SKI 1008-treated groups were significantly shorter than those for the ganciclovir-treated groups ($P < 0.01$). However, no animal in all the treatment groups survived on the last evaluation day (day 30). Like the results obtained from the experiment in normal BALB/c mice, the in vivo anti-MCMV efficacy of SKI 1008 in SCID mice was lower than that of ganciclovir based on the delay in mean survival time.

4. Discussion

SKI 1008, an isosteric analog of PMEG, was evaluated for its in vitro antiviral activity against HSV-1, HSV-2, MCMV, and HCMV, its in vivo antiviral efficacy against MCMV in mice, and its cytotoxicity in uninfected cells using the MTT assay.

The in vitro antiviral effect of SKI 1008 on the replication of HSV-1 and HSV-2 was compared with that of acyclovir. It has already been demonstrated that the monoethyl esters of acyclic nucleoside phosphonates are highly active against HCMV, but are not very active against HSV (Barnard et al., 1993). As we expected, the anti-HSV activity of SKI 1008 against TK^+ strains was much lower than that of acyclovir; however, the similar antiviral activity of SKI 1008 against TK^+ and TK^- strains of HSV suggests that the antiviral mode of action of SKI 1008 should be independent of HSV-encoded TK.

Although the in vitro anti-MCMV activity of SKI 1008 was 3-fold more potent than that of ganciclovir, the anti-HCMV activity of SKI 1008 was comparable to that of ganciclovir, as determined by plaque reduction assay. This discrepancy seems to be related to the different sensitivity between mouse and human host cell system (Tyms et al., 1981; Smee et al., 1989). In this study, SKI 1008 was more selective than PMEG in inhibiting the replication of MCMV and HCMV in vitro.

We evaluated the antiviral efficacy of SKI 1008 in normal BALB/c mice infected with MCMV, which has been used as a model for human CMV infections and in vivo anti-CMV agents evaluation (Hudson, 1979; Kern, 1988, 1991). We also assessed the

antiviral efficacy of SKI 1008 in the MCMV/SCID mouse model (Neyts et al., 1992; Smee et al., 1992), which is suitable for evaluating the potential of antivirals in the therapy of CMV infection in the immunocompromised patients. A major problem encountered in the current ganciclovir treatment of HCMV infection in immunocompromised patient is the relapse of disease after cessation of therapy (Laskin et al., 1987).

Even though SKI 1008 exhibited greater in vitro anti-MCMV activity, the in vivo anti-MCMV efficacy of SKI 1008 was lower than that of ganciclovir, as monitored by the survival rate and mean survival day. The reason for the discrepancy between the in vitro and in vivo anti-MCMV activity of SKI 1008 is not yet clear. Although there is no direct evidence at present, pharmacokinetics may play a role in the poor efficacy of SKI 1008 relative to that of ganciclovir.

In the MCMV/SCID model system, ganciclovir achieved better efficacy than SKI 1008, although ganciclovir was not able to prevent the death caused by MCMV and to suppress MCMV replication in organs after cessation of therapy; virus titers of ganciclovir-treated group increased up to the levels observed in the SKI 1008-treated group on day 14 postinfection (data not shown). Similar results have been reported previously (Neyts et al., 1992; Smee et al., 1992).

In conclusion, the results obtained from these antiviral efficacy assays do not encourage us to develop SKI 1008 as a clinically useful anti-HCMV agent. However, this study provides some insight into the structural requirements for the rational design of analogs of PMEG.

References

Alford, C.A. and Britt, W.J. (1990) Cytomegalovirus. In: B.N. Fields and D.M. Knipe (Eds.), Fields Virology, 2nd edn. Raven Press, New York, pp. 1981–2010.

Andrei, G., Snoeck, R., Schols, D., Goubau, P., Desmyter, J. and De Clercq, E. (1991) Comparative activity of selected antiviral compounds against clinical isolates of human cytomegalovirus. Eur. J. Clin. Microbiol. Infect. Dis. 10, 1026–1033.

Balfour, H.H. (1990) Management of cytomegalovirus disease with antiviral drugs. Rev. Infect. Dis. 12, S849–S860.

Barnard, D.L., Huffman, J.H., Sidwell, R.W. and Reist, E.J. (1993) Selective inhibition of cytomegaloviruses by 9-(3′-ethylphosphono-1′-hydroxymethyl-1′-propyloxymethyl)guanine. Antiviral Res. 22, 77–89.

Bronson, J.J., Kim, C.U., Ghazzouli, I., Hitchcock, M.J.M., Kern, E.R. and Martin, J.C. (1989) Synthesis and antiviral activity of phosphonylmethoxyethyl derivatives of purine and pyrimidine bases. In: J.C. Martin (Ed.), Nucleotide Analogues as Antiviral Agents. American Chemical Society, Bethesda, MD, pp. 72–87.

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., Miner, R.C., Busch, D.F., Follansbee, S.E., Gullet, J., Mehalko, S.G., Gordon, S.M., Owen, W.F., Jr., 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.

Hudson, J.B. (1979) The murine cytomegalovirus as a model for the study of viral pathogenesis and persistent infections. Arch. Virol. 62, 1–29.

Kern, E.R. (1988) Animal models as assay systems for the development of antivirals. In: E. De Clercq and R.T. Walker (Eds.), Antiviral Drug Development. A Multidisciplinary Approach. Plenum Press, New York, pp. 149–172.

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

Kim, C.U., Misco, P.F., Luh, B.Y., Hitchcock, M.J.M., Ghazzouli, I. and Martin, J.C. (1991) A new class of acyclic phosphonate nucleotide analogues-phoshonate isosteres of acyclovir and ganciclovir monophosphates as antiviral agents. J. Med. Chem. 34, 2286–2294.

Kim, D.-K., Kim, Y.-W. and Kim, K.H. (1994) Synthesis and anti-HCMV activity of 9-[[(ethoxyhydroxyphosphinyl)methoxy]methoxy]guanine, an isostere of PMEG monoethyl ester. Bioorg. Med. Chem. Lett. 4, 2241–2244.

Koretz, H. and Collaborative DHPG treatment study group (1986) Treatment of serious cytomegalovirus infections with 9-(1,3-dihydroxy-2-propoxymethyl)guanine in patients with AIDS and other immunodeficiencies. New Engl. J. Med. 314, 801–805.

Laskin, O.L., Stahl-Bayliss, C.M., Kalman, C.M. and Rosecan L.R. (1987) Use of ganciclovir to treat serious cytomegalovirus infections in patients with AIDS. J. Infect. Dis. 155, 323–327.

Meyers, J.D. (1991) Prevention and treatment of cytomegalovirus infection. Ann. Rev. Med. 42, 179–187.

Neyts, J., Balzarini, J., Naesens, K. and De Clercq, E. (1992) Efficacy of (*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine and 9-(1,3-dihydroxy-2-propoxymethyl)guanine for the treatment of murine cytomegalovirus infection in severe combined immunodeficiency mice. J. Med. Virol. 37, 67–71.

Raju, N., Smee, D.F., Robins, R.K. and Vaghefi, M.M. (1989) Synthesis and biological properties of purine and pyrimidine 5′-deoxy-5′-(dihydroxyphosphinyl)-β-D-ribofuranosyl analogs of AMP, GMP, IMP, and CMP. J. Med. Chem. 32, 1307–1313.

Reist, E.J., Sturm, P.A., Pong, R.Y. and Sidwell, R.W. (1988) Synthesis of phosphonic acid analogs of acyclovir (ACV) and ganciclovir (DHPG). Nucleosides Nucleotides 8, 919–922.

Reist, E.J., Sturm, P.A., Pong, R.Y., Tanga, M.J. and Sidwell, R.W. (1989) Synthesis of acyclonucleoside phosphonates for evaluation as antiviral agents. In: J.C. Martin (Ed.), Nucleotide Analogues as Antiviral Agents. Maple Press, York, ACS Symposium Series, pp. 17–34.

Sidwell, R.W., Huffman, J.H., Barnard, D.L. and Reist, E.J. (1988) Effect of phosphonic acid analogs of acyclovir and ganciclovir on in vitro cytomegalovirus infections. Nucleosides Nucleotides 8, 1159–1160.

Smee, D.F., Colletti, A., Alaghamandan, H.A. and Allen, L.B. (1989) Evaluation of continuous cell lines in antiviral studies with murine cytomegalovirus. Arch. Virol. 107, 253–260.

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, interferon, and bropirimine. Antimicrob. Agents Chemother. 36, 1837–1842.

Snoeck, R., Sakuma, T., De Clercq, E., Rosenberg, I. and Holy, A. (1988) (*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine, a potent and selective inhibitor of human cytomegalovirus replication. Antimicrob. Agents Chemother. 32, 1839–1844.

Tyms, A.S., Scamans, E.M. and Naim, H.M. (1981) The in vitro activity of acyclovir and related compounds against cytomegalovirus infections. J. Antimicrob. Chemother. 8, 65–72.