ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Jan. 1995, p. 56–60 Vol. 39, No. 1
0066-4804/95/$04.00+0
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In Vivo Antiherpesvirus Activity of N-7-Substituted Acyclic Nucleoside Analog 2-Amino-7-[(1,3-Dihydroxy-2-Propoxy)Methyl]Purine

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Received 20 May 1994/Returned for modification 24 June 1994/Accepted 11 October 1994

The efficacy of 2-amino-7-[(1,3-dihydroxy-2-propoxy)methyl]purine (S2242) was evaluated in several animal models for herpesvirus infections. Compound S2242 was more effective than acyclovir (i) when administered subcutaneously in a model for herpes simplex virus type 1 (HSV-1)-induced mortality in immunocompetent mice and (ii) when applied topically to hairless (*hr*/*hr*) mice that had been infected intracutaneously with HSV-2. In SCID (severe combined immune deficient) mice that had been infected with a thymidine kinase-deficient HSV-1 strain, S2242 (administered subcutaneously at a dosage of 50 mg/kg/day) completely protected against virus-induced mortality whereas foscarnet was less effective and acyclovir had no or little protective effect. Compound S2242 was far more effective than ganciclovir in preventing or delaying murine cytomegalovirus-induced mortality in immunocompetent and SCID mice. The compound was more effective when a given dose was fractionated and administered on subsequent days than when this dose was administered in one single injection. A 5-day treatment course with S2242 (10 and 50 mg/kg/day) for newborn mice that had been infected with a lethal dose of murine cytomegalovirus suppressed virus-induced mortality. Compound S2242 had no inhibitory effect on the growth of weanling (at 50 mg/kg for 5 days) and 3- to 4-week-old mice (at doses of 50 to 200 mg/kg for 6 weeks). However, akin to ganciclovir, compound S2242 significantly reduced testicle weight, testicle morphology, and spermatogenesis.

A major goal of the search for new antiviral drugs is the development of antiherpesvirus agents that have resistance and toxicity profiles that are different from those of the currently available drugs. Little attention has so far been paid to the synthesis of purine nucleoside analogs with the side chain substituted at the purine N-7 position. Recently we reported on the synthesis of a series of N-7-substituted acyclic nucleoside analogs (6). Of this class of compounds, 2-amino-7-[(1,3-dihydroxy-2-propoxy)methyl]purine (S2242) was shown to be a potent inhibitor of herpesvirus replication in vitro. Of special interest is the potent activity of the compound against cytomegalovirus (CMV) and thymidine kinase-deficient (TK^-) strains of herpes simplex virus (HSV) and varicella-zoster virus (7). We report here on the antiherpesvirus activity of compound S2242 in several animal models for herpesvirus infection.

MATERIALS AND METHODS

Compounds. Acyclovir (ACV) (Zovirax) was obtained from Wellcome Research Laboratories (Aalst, Belgium), ganciclovir (GCV) (Cymevene) was obtained from Sarva-Syntex (Brussels, Belgium), and foscarnet (PFA) (Foscavir) was obtained from Astra Pharmaceutical Products, Inc. (Södertalje, Sweden). The synthesis of 2-amino-7-[(1,3-dihydroxy-2-propoxy)methyl]purine (compound S2242) has been reported elsewhere (6).

Mice. The animals used throughout the experiments were (i) NMRI (Naval Medical Research Institute) mice, which were housed under conventional conditions during the experiments; (ii) hairless (*hr*/*hr*) mice (weight, 15 to 20 g) that were bred at the Rega Institute by backcrossing and intercrossing of the homozygous parents, which were also housed under conventional conditions during the experiments; (iii) SCID (severe combined immune deficient) mice (C.B-17 *scid*/*scid* inbred strain bred at the Rega Institute under germ-free conditions); and (iv) nude (*nu*/*nu*) mice (Charles River Breeding, Sulzfeld, Germany) that were housed under specific pathogen-free conditions during the experiments.

Viruses. The origins of the virus strains have been described previously: for murine CMV (MCMV), see reference 8; for HSV type 1 (HSV-1) (strain KOS) and HSV-2 (strain 196), see reference 2; and for TK^- HSV-1 (strain VMW-1837), see references 1 and 3). The VMW-1837 strain was isolated from an immunosuppressed patient with a chronic HSV-1 infection that had become resistant to ACV treatment (14). Another HSV-1 strain, designated Hu10, which was resistant to both ACV and PFA, was isolated from a bone marrow transplant recipient; strain Hu3 that has been isolated from the same patient was susceptible to ACV and PFA (12, 13).

i.p. HSV-1 infection of NMRI mice. Immunocompetent NMRI mice (±13 g) were inoculated intraperitoneally (i.p.) with HSV-1 (KOS) at 10^3 PFU per 0.2 ml per mouse. The test compounds were administered subcutaneously (s.c.) in 0.2-ml volumes once a day, for 5 consecutive days, starting 1 h after infection.

i.p. TK^- HSV-1 infection in SCID mice. SCID mice (weight, ±12 g) were infected i.p. with 10^4 PFU per 0.2 ml of TK^- HSV-1 (strain VMW-1837). The test compounds were administered s.c. in 0.2-ml volumes once a day, for 5 consecutive days, starting 1 h after the infection.

i.c. HSV-2 infection in hairless mice. Hairless mice were inoculated intracutaneously (i.c.) at the lumbosacral area (by scratching the skin with a scarificator) with HSV-2 (strain 196) at $10^{3.7}$ PFU per 0.05 ml per mouse. As a rule the mice were treated for 5 days, starting 1 h after virus infection, with the test compounds, which were applied topically at the indicated concentrations in dimethyl sulfoxide (four times daily) in a volume of 0.05 ml over an area of 1.5 cm^2. Alternatively, compound S2242 was introduced in plasters containing 100 mg of a galenic formulation (Eutanol G-Arlacel 186-Vaseline, 1:1:8), which were applied to the infected area 3 h after infection and which were left on for 4 days. The mice were monitored daily for the development of herpetic skin lesions and mortality.

i.c. HSV-1 infection in nude mice. Nude mice weighing 12 g were inoculated i.c. with HSV-1 strain Hu10 or Hu3 at 6×10^3 PFU per 0.05 ml. The topical treatment was based on either ACV or compound S2242 (5% ointment in dimethyl sulfoxide) 4 times a day for 5 consecutive days.

Infection of NMRI and SCID mice with MCMV. NMRI mice weighing 9 to 10 g were inoculated i.p. with 10^5 PFU per 0.2 ml of MCMV. Four- to five-day-old NMRI mice were infected i.p. with 10^3 PFU per 0.05 ml of MCMV. SCID mice (about 5 weeks old; weight ± 18 g) were infected in a way similar to that for the NMRI mice, except for the conditions of the experiment presented in Table 6 (see Table 6, footnote *a*).

Titration of MCMV in several organs of SCID mice. Spleens, lungs, and brains from two animals were removed aseptically at each time point, and the titers of MCMV in tissue homogenates (10% wt/vol) of primary murine embryo fibroblast cells were determined as described previously (8).

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TABLE 1. Effect of treatments with compounds S2242 and ACV on HSV-1-induced mortality in NMRI mice

Treatment[a]	Dosage (mg/kg/day)	No. of survivors[b,c]	MDD (mean ± SD)[c]
None (control)		3	6.9 ± 1.2
S2242	100	10***	
	50	10***	
	20	10***	
	5	3	9.2 ± 1.8**
	1	1	8.0 ± 1.5
ACV	100	5	11.6 ± 1.9***
	50	6	12.2 ± 4.2*
	20	0	8.2 ± 1.2*
	5	2	7.2 ± 0.8
	1	2	6.6 ± 0.9

[a] s.c. treatment was started on day 0 postinfection and was continued once daily for the next 4 days.
[b] Values are numbers of survivors from a total of 20 mice (control) or 10 mice (S2242 and ACV).
[c] *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$ for comparison with the control condition.

Toxicology. The potential subacute toxicity of compound S2242 was evaluated by (i) determination of the body weight of male NMRI mice treated (s.c.) during a 14-day period (one injection per day) with 100 mg of compound S2242 or GCV per kg of body weight or of HSV-1-infected mice treated (s.c.) during a 6-week period with 200 mg of compound S2242 or GCV per kg and (ii) determination of the testicle weight of noninfected mice 4 weeks after a 14-day treatment period with 100 mg of compound S2242 or GCV per kg or 1 day after a 6-week treatment period for HSV-1-infected mice with 50 to 200 mg of the compounds per kg.

Histology. Organs (spleen, testis, epididymis, bone marrow, thymus, kidney, liver) of drug-treated animals (50 to 200 mg/kg during a 42-day period) were fixed in Carnoy's fixative. Paraffin sections were stained with hematoxylin and eosin.

Statistical analysis. The significance of the number of survivors was calculated using the χ^2 test with Yates' correction. The statistical significance of the mean day of death (MDD) was assessed by the Student t test.

RESULTS

Effect of systemic treatment with compound S2242 or ACV in NMRI mice infected i.p. with HSV-1. As is shown in Table 1, treatment of HSV-1-infected NMRI mice with S2242 at a dosage of 20 mg/kg/day for 5 consecutive days completely protected the animals against virus-induced mortality. Some protective activity (delay in MDD) was still observed at a dosage of 5 mg/kg/day.

Effect of topical treatment with compound S2242 or ACV in hairless mice infected i.c. with HSV-2. Hairless (*hr*/*hr*) mice that had been infected i.c. with HSV-2 developed lesions at the site of inoculation about 3 days postinfection, which gradually evolved to paralysis of the hind limbs and finally to death (Table 2). All animals that had been treated topically with a 5% ointment of S2242 four times daily for the first 5 days postinfection remained free of lesions and survived. Ointments containing 0.2 or 1% S2242 also afforded some protective activity. Since oral administration by licking after topical administration cannot be excluded, compound S2242 was applied in plasters. Also, under this experimental design compound S2242 (5%) proved highly effective (i.e., 13 of 15 mice were survivors compared with 4 of 15 for the control, $P < 0.02$).

Effect of systemic treatment with compound S2242, PFA, or ACV on TK$^-$ HSV-1-induced mortality in SCID mice. In SCID mice, i.p. TK$^-$ HSV-1 infection resulted in 100% mortality within 10 days (Table 3). Animals that were treated during the first week postinfection with S2242 at dosages of 25 or 50 mg/kg/day survived for more than 5 months (at which time the experiment was stopped). As could be expected, ACV conferred only moderate activity (i.e., delay in MDD) in this model. PFA offered protective activity at dosages of 200 to 1,000 mg/kg/day.

TABLE 2. Effect of topical treatments with compounds S2242 and ACV[a] on mortality of hairless (*hr*/*hr*) mice infected i.c. with HSV-2

Treatment	Dosage (% in DMSO)	No. of survivors[b,c]	MDD (mean ± SD)[c]
None (control; DMSO)		0	5.5 ± 0.6
S2242	5	10**	
	1	3*	12.0 ± 2.8**
	0.2	0	8.3 ± 1.3**
ACV	5	0	10.1 ± 3.7**

[a] Started on day 0 at 2 h after infection and continued 4 times daily for the next 4 days. DMSO, dimethyl sulfoxide.
[b] Values are numbers of survivors from a total of 14 mice (control) or 10 mice (S2242 and ACV).
[c] *, $P < 0.05$; **, $P < 0.001$ for comparison with the control condition.

Effect of topical treatment with compound S2242 or ACV on wild-type and ACV-PFA-resistant HSV-1 infections in nude mice. Application of either 5% ACV or 5% S2242 (ointment) significantly delayed wild-type HSV-1 (strain Hu3)-induced mortality, and the S2242 treatment significantly increased the number of survivors (Table 4). Since the ACV-PFA-resistant HSV-1 (strain Hu10) does not cause mortality, the mean day of lesion initiation and the number of mice developing lesions were used as markers of drug efficacy. A 5-day treatment course with 5% ACV ointment did not result in any beneficial effect. In contrast, a similar treatment schedule with S2242 significantly delayed the mean day of lesion initiation and tended to reduce the number of animals developing lesions.

Effect of treatment with compound S2242 or GCV on MCMV-induced mortality in NMRI mice. Compound S2242 administered at 5 to 25 mg/kg/day for 5 consecutive days, starting on the day of infection, resulted in 90% (5 mg/kg/day) to 100% (25 mg/kg/day) survivors. Even one dose of S2242 at 50 mg/kg, administered on the day of infection, resulted in

TABLE 3. Effect of compounds S2242, ACV, and PFA on TK$^-$ HSV-1-induced mortality in SCID mice

Treatment[a]	Dosage (mg/kg/day)	No. of survivors[b,c]	MDD (mean ± SD)[c]
None (control)		0	9.3 ± 2.0
S2242	50	10***	>120***[d]
	25	4**	22.8 ± 7.8***
ACV	100	1	11.5 ± 1.4**
	50	2	13.7 ± 5.4*
	25	0	11.3 ± 1.8**
PFA	1,000	9***	10
	500	7***	16.0 ± 9.0
	200	3*	11.0 ± 1.1
	100	0	9.3 ± 1.4

[a] Started 2 h postinfection and continued once daily for the next 4 days.
[b] Values are numbers of survivors from a total of 25 mice (control) or 10 mice (S2242, ACV, and PFA).
[c] *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$ for comparison with the control.
[d] The mice survived for more than 120 days after infection.

TABLE 4. Effect of topical treatments with compounds S2242 and ACV on wild-type (Hu3) and ACV- and PFA-resistant (Hu10) HSV-1 infections in nude mice

Treatment[a]	MDD for Hu3[b,c]	MDLI for Hu10[c,d]
None (control)	7.7 ± 1.1 (0/9)	5.7 ± 1.7 (8/8)
S2242	23.5 ± 9.5* (6/8)**	13 ± 3* (4/8)[e]
ACV	20.2 ± 7.6** (4/8)	6.6 ± 1.8 (8/8)

[a] Administered topically as a 5% ointment four times daily for 5 consecutive days, starting on the day of infection.
[b] Values are means ± standard deviations. Values in parentheses are numbers of survivors per numbers of mice tested.
[c] *, $P < 0.01$ and **, $P < 0.05$ for comparison with the control condition.
[d] MDLI, mean day of lesion initiation. Values are means ± standard deviations. Values in parentheses are numbers of mice developing lesions per numbers of mice tested.
[e] Two of the four mice that developed lesions had only very small lesions at the inoculation site.

TABLE 5. Effect of prolonged treatments with compounds S2242 and GCV on MCMV-induced mortality in SCID mice

Treatment[a]	Dosage (mg/kg/day)	MDD[b]
None (control)		7.2 ± 1.9
S2242	100	101.8 ± 9.2*[c]
	20	41.6 ± 1.7*
GCV	100	26.2 ± 5.8*
	20	19.4 ± 0.5*

[a] Mice received the compounds for 5 consecutive days every week (five or six mice per group).
[b] *, $P < 0.001$ compared with the control.
[c] No virus could be recovered from the spleens, brains, or lungs at the time of death.

100% survival. GCV, administered in 5 dosages of 25 mg/kg/day, resulted in 75% survival (data not shown).

Four- to five-day-old mice that had been inoculated with a lethal dose of MCMV all died at about 4 days postinfection. However, when treated with S2242 at dosages of 10 or 50 mg/kg/day for the first 5 days postinfection, all animals (five of five) survived the infection. When treated with 2 mg/kg/day, only one of five survived but the MDD (mean ± standard deviation) was increased from 3.6 ± 0.5 to 7.2 ± 0.4 days, compared with the control ($P < 0.001$). Moreover, these animals grew as normally as did uninfected untreated animals. No toxicity of S2242 was observed at dosages of up to 50 mg/kg/day in noninfected animals, as monitored by following the increase in body weight (data not shown).

Effect of treatment with compound S2242 or GCV on MCMV infection in SCID mice. In MCMV-infected SCID mice treated with S2242 or GCV at a dosage of 20 mg/kg/day, the onset of virus replication in the lungs and spleens of mice was delayed (Fig. 1) and virus titers were lower than in control animals.

SCID mice that had been infected i.p. with MCMV were treated s.c. for 5 consecutive days every week (Table 5). All GCV-treated animals died within 1 month. The virus that was recovered from the lungs of the GCV-treated animals proved as susceptible to the drug as the wild-type virus, indicating that the animals did not die from a GCV-resistant virus but rather from a disseminated infection (data not shown). Animals that had been treated with S2242 at a dosage of 20 mg/kg/day survived twice as long as mice that had been treated with a similar dose of GCV. The virus that was recovered from the lungs of these animals also proved as susceptible to the compound as the wild-type virus (data not shown). A higher dosage of S2242, i.e., 100 mg/kg/day, resulted in survival period longer than 3 months. Of this group, one animal died with a thymoma (which is often observed in long-living SCID mice), two had unexplained petechiae on their livers, and no autopsies could be performed on the other animals.

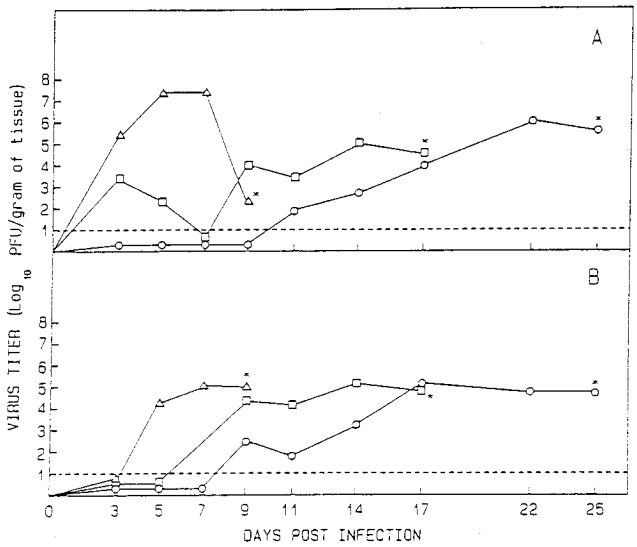

FIG. 1. Effects of GCV and compound S2242 on MCMV replication in the spleens (A) and lungs (B) of SCID mice. Treatment was initiated 2 h after infection and was continued for the next 4 days. Symbols: △, untreated; □, GCV; ○, compound S2242; *, no survivors.

We next evaluated whether prolonged treatment with a total weekly dose of 100 mg of S2242 or GCV per kg was more efficient when this dose was divided into several injections than when given as a single weekly injection (data not shown). The MCMV-infected SCID mice (MDD for control mice, 10.4 ± 3.7) received the drugs in either one single dose (100 mg/kg), two doses (50 mg/kg each), three doses (33 mg/kg each), or five doses (20 mg/kg each). Irrespective of the treatment schedule, all GCV-treated animals succumbed within 22 days after the infection. In contrast, S2242 was more effective when administered in 5 doses a week (MDD, 68 ± 21; $P < 0.001$) than when administered in one (MDD, 29 ± 3.5; $P < 0.001$), two (MDD, 39 ± 7; $P < 0.001$), or three (MDD, 37 ± 4; $P < 0.001$) doses a week.

An experiment to determine how long the start of treatment with S2242 or GCV could be delayed in a slowly progressing MCMV infection in SCID mice was set up (Table 6). SCID mice were infected with a low inoculum of MCMV so that they died at about 16 days postinfection. When treatment with either GCV or S2242 was initiated on day 0 postinfection, the animals survived for 5 and 7 to 8 weeks, respectively. However, if the start of treatment with GCV was delayed until the time that extensive virus replication was observed in various organs, the compound lost part (if treatment started on day 4 or 8 postinfection) or all (if treatment started on day 13 postinfection) of its effectiveness. In contrast, if the initiation of treatment with S2242 was delayed until day 13 postinfection, a strong antiviral activity was still observed.

Toxicology. The increase in body weight of 3- to 4-week-old NMRI mice that had received 100 mg of S2242 or GCV per kg over a 14-day period was comparable to that of untreated mice (data not shown). Similarly, HSV-1-infected NMRI mice that

TABLE 6. Effect of delayed start of treatments with compounds S2242 and GCV on lethal MCMV infection in SCID mice

Start of treatment[a] (postinfection day)	Compound	MDD[b]
0 (control)	None	16.4 ± 3.1
0	S2242	53.5 ± 10.1**[c]
0	GCV	35.1 ± 2.2**[c]
4	S2242	38.8 ± 5.7**[d]
4	GCV	31.7 ± 12.8**[d]
8	S2242	49.2 ± 3.8**[c]
8	GCV	28.7 ± 2.8**[c]
13	S2242	44.5 ± 9.8**[c]
13	GCV	23.8 ± 11.1*[c]

[a] Compound was administered s.c. three times a week on alternating days (starting on the indicated day) at 50 mg/kg/day. Mice were infected i.p. with 5×10^2 PFU of MCMV. Each treatment group consisted of 9 mice; the control group consisted of 21 mice.

[b] *, $P < 0.02$ and **, $P < 0.001$ for comparison with the control.

[c] $P < 0.001$ for comparison of the two treatment groups.

[d] $P > 0.05$ for comparison of the two treatment groups.

received 50 to 200 mg of S2242 or GCV per kg for 6 weeks grew up normally compared with untreated animals (data not shown). When the testicle weights of HSV-1-infected mice that had received 50 to 200 mg of S2242 or GCV per kg over a 6-week period (data not shown) and those of noninfected mice that had received 100 mg of the compounds per kg for 14 days were determined (Fig. 2), a marked weight reduction was observed for the drug-treated groups. All mice of the GCV- and compound S2242-treated groups displayed atrophy of the testicular germinal epithelium, leaving alone Sertoli cells and the basement membrane (Sertoli Cell Only syndrome). ACV at 200 mg/kg caused only mild spermatogenic disorders. Correlated with this, epididymal channels were free of spermatozoa (GCV and compound S2242) or showed a slight decrease thereof (ACV). Most of the mice in the ACV-treated group and individual mice in the GCV-dosed group exhibited mild atrophy of renal tubules, whereas the S2242-treated animals did not. In the liver, single cell or cell group necrosis was detected in both groups treated with 50 to 200 mg of ganciclovir and S2242 per kg and in the group treated with 200 mg of acyclovir per kg.

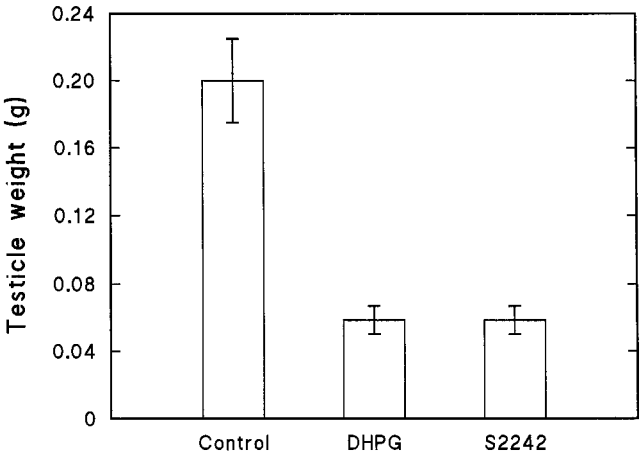

FIG. 2. Effects of compound S2242 and GCV on testicle weight after a 14-day treatment at 100 mg/kg/day. Testicle weight was determined 4 weeks after the end of treatment. Each group consisted of 10 animals. DHPG, GCV; control, uninfected mice.

DISCUSSION

The results of our experiments pertain to the efficacy of S2242 in the treatment of various experimental in vivo models of herpesvirus infections. The compound proved markedly effective in (i) the systemic treatment of lethal disseminated HSV-1 infection in immunocompetent mice, (ii) the topical treatment of i.c. HSV-2 infection in hairless mice, (iii) the systemic treatment of TK^- HSV-1 infections in SCID mice, and (iv) the topical treatment of i.c. infections in nude mice with a clinical HSV-1 strain that was resistant to both PFA and ACV.

Also, when evaluated in a model for lethal MCMV infection in NMRI mice, S2242 proved effective. Although the MCMV model in immunocompetent mice may have some predictive value for the clinical setting, it does not fully mimic the situation in the immunocompromised host, where the CMV disease progresses after the discontinuation of antiviral therapy. Also, in patients the virus may become resistant to GCV and/or PFA following long-term treatment, and some patients fail to respond to maintenance therapy (5, 10). In immunocompetent mice, the MCMV disease does not relapse after the termination of successful antiviral therapy (3). In contrast, in SCID mice antiviral therapy alone does not eradicate the virus, and as the virus persists in a virulent state, disease progression may occur after the termination of antiviral therapy. In the MCMV-SCID mouse model, compound S2242 proved much more potent than GCV when administered over a long period of time. The virus that was isolated from the lungs of the animals that ultimately died while under therapy with GVC or S2242 (at the lower dosage) proved as susceptible to each compound as the wild-type virus. This indicates that over a 3-month treatment period with S2242, at least in mice infected with MCMV, resistance does not readily develop.

A strategy that is receiving increasing attention as a prophylactic measure in transplant recipients is the so-called preemptive therapy with GCV. Asymptomatic transplant recipients are examined at regular intervals after transplantation for the presence of CMV in bronchoalveolar lavage fluid or blood (11). As soon as CMV is detected, GCV therapy is started. By using the MCMV-SCID model, we tried to create a situation (with a low level of virus) in which the disease proceeded slowly, so that treatment could be started at different days postinfection over a relatively long period of time. It appeared that the initiation of therapy with S2242 could be delayed for up to 13 days after infection, a time when extensive virus replication was already observed. GCV proved less effective under these test conditions.

Treatment with S2242 was also highly effective in preventing MCMV-induced mortality in 4- to 5-day-old NMRI mice. Moreover, no growth retardation was observed, even at doses that were 25-fold higher than that affording 100% protection against virus-induced mortality. Finally, at doses exceeding by far the antivirally active doses, compound S2242 had no inhibitory effect on the growth of 3- to 4-week-old noninfected (14-day treatment) or HSV-1-infected (6-week treatment) NMRI mice. However, akin to GCV (14), the compound appeared to have a major effect on testicle weight and histology. Besides the effect on testicle function, no major histological abnormalities were observed in the other organs obtained from the mice treated with the indicated doses.

In conclusion, S2242 appears to be a remarkably potent inhibitor of herpesvirus infections in vivo. In particular (i) it is more potent than ACV in the s.c. treatment of systemic HSV-1 infection and in the topical treatment of i.c. HSV-2 infection, (ii) it is active against infections with TK^- and double-resistant (PFA- and ACV-resistant) strains of HSV-1, and (iii) it is superior to GCV in the treatment of MCMV infections in several clinically relevant models.

ACKNOWLEDGMENTS

We thank Willy Zeegers for expert technical assistance and Christiane Callebaut for fine editorial help.

These investigations were supported by the Belgian Fonds voor Geneeskundig Wetenschappelijk Onderzoek (project number 3.0026.91). J. Neyts is a postdoctoral research assistant from the Belgian National Fund for Scientific Research (NFWO).

REFERENCES

1. **De Clercq, E.** 1987. Virus-drug resistance: thymidine kinase-deficient (TK^-) mutants of herpes simplex virus. Therapeutic approaches. Ann. Ist. Super. Sanita **23:**841–848.
2. **De Clercq, E., J. Descamps, G. Verhelst, R. T. Walker, A. S. Jones, P. F. Torrence, and D. Shugar.** 1980. Comparative efficacy of different antiherpes drugs against different strains of herpes simplex virus. J. Infect. Dis. **141:** 563–574.
3. **De Clercq, E., A. Holy, I. Rosenberg, T. Sakuma, J. Balzarini, and P. C. Maudgal.** 1986. A novel selective broad-spectrum anti-DNA virus agent. Nature (London) **323:**464–467.
4. **Faulds, D., and R. C. Heel.** 1990. Ganciclovir, a review of its antiviral activity, pharmacokinetic properties and therapeutic efficacy in cytomegalovirus infections. Drugs **39:**597–638.
5. **Holland, G. M., M. J. Sakamoto, O. Hardy, and Y. Sidikaro.** 1986. Treatment of cytomegalovirus retinopathy in patients with acquired immunodeficiency syndrome. Arch. Ophthalmol. **104:**1794–1800.
6. **Jähne, G., H. Kroha, A. Müller, M. Helsberg, I. Winkler, G. Gross, and T. Scholl.** 1994. Regioselective synthesis and antiviral activity of purine nucleoside analogues with acyclic substituents at N7. Angew. Chem. **106:**603–605 (Int. Ed. Engl. **33:**562–563, 1994).
7. **Neyts, J., G. Andrei, R. Snoeck, G. Jähne, I. Winkler, M. Helsberg, J. Balzarini, and E. De Clercq.** 1994. The N-7-substituted acyclic nucleoside analog 2-amino-7-[(1,3-dihydroxy-2-propoxy)methyl]purine is a potent and selective inhibitor of herpesvirus replication. Antimicrob. Agents Chemother. **38:**2710–2716.
8. **Neyts, J., J. Balzarini, L. Naesens, and E. De Clercq.** 1992. Efficacy of (S)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine and 9-(1,3-dihydroxy-2-propoxymethyl)guanine in the treatment of murine cytomegalovirus infection in severe combined immunodeficiency mice. J. Med. Virol. **37:**67–71.
9. **Neyts, J., H. Sobis, R. Snoeck, M. Vandeputte, and E. De Clercq.** 1993. Efficacy of (*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine and 9-(1,3-dihydroxy-2-propoxymethyl)guanine in the treatment of intracerebral murine cytomegalovirus infections in immunocompetent and immunodeficient mice. Eur. J. Clin. Microbiol. Infect. Dis. **12:**269–279.
10. **O'Donnell, J. J., M. A. Jacobson, and J. Mills.** 1987. Development of cytomegalovirus (CMV) retinitis in patients with AIDS during ganciclovir therapy for CMV colitis. N. Engl. J. Med. **316:**1607–1608.
11. **Schmidt, G. M.** 1992. Prophylaxis of cytomegalovirus infection after bone marrow transplantation. Semin. Oncol. **19**(Suppl. 7)**:**S20–S26.
12. **Snoeck, R., G. Andrei, E. De Clercq, M. Gerard, M. Clumeck, G. Tricot, and C. Sadzot-Delvaux.** 1993. A new topical treatment for resistant herpes simplex infections. N. Engl. J. Med. **329:**968–969.
13. **Snoeck, R., G. Andrei, M. Gérard, A. Silverman, A. Hedderman, J. Balzarini, C. Sadzot-Delvaux, G. Tricot, N. Clumeck, and E. De Clercq.** 1994. Successful treatment of progressive mucocutaneous infection due to acyclovir- and foscarnet-resistant herpes simplex virus with (S)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine (HPMPC). Clin. Infect. Dis. **18:**570–578.
14. **Vinckier, F., M. Boogaerts, D. Declerck, and E. De Clercq.** 1987. Chronic herpetic infection in an immunocompromised patient. Report of a case. J. Oral Maxillofac. Surg. **45:**723–728.