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Short communication

Efficacy of ganciclovir and cidofovir against human cytomegalovirus replication in SCID mice implanted with human retinal tissue

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Abstract

For immunocompromised hosts, human cytomegalovirus (CMV) can be a serious problem. For evaluation of antivirals used to treat CMV retinitis, we have used severe combined immunodeficient mice as hosts for human retinal tissue implanted in the eye and subsequently infected with CMV. Treatment with ganciclovir or cidofovir resulted in a significant suppression of CMV replication.

Keywords: Ganciclovir; Cidofovir; Human cytomegalovirus; SCID-hu mice

Severe human cytomegalovirus (CMV) end organ disease occurs most frequently in patients infected with human immunodeficiency virus (HIV) or those undergoing organ transplantation (Alford and Britt, 1993; Gershon et al., 1997). Prior to the use of HAART, one of the most common AIDS-related CMV diseases reported was CMV retinitis. This disease, first reported as a complication of AIDS in 1982, is characterized by full-thickness retinal necrosis and edema and if left untreated, can result in severe visual impairment or blindness (Jacobson, 1997). As portions of the retina damaged by CMV do not regain function, antiviral therapy can be used only to prevent further damage. Currently, five antiviral drugs are available for treatment of CMV retinitis. Ganciclovir (GCV), valganciclovir, foscarnet, and cidofovir (CDV) inhibit the activity of the viral DNA polymerase essential for replication of HCMV (Cocohoba and McNicholl, 2002; Crumpacker, 1996; Lea and Bryson, 1996; Wagstaff and Bryson, 1994) while fomivirsen is an antisense oligonucleotide that inhibits production of CMV proteins (Perry and Balfour, 1999). In many patients, therapy has only slowed the progression of the disease and in others resistance has developed (Emery, 1993; van der Meer et al., 1996). Additionally, long-term therapy has resulted in serious, adverse events that required cessation of treatment (Jacobson et al., 1999). Taken together, these problems clearly indicate the need for improved therapeutics for CMV infections.

As part of the development of new antiviral therapies, there is a need for in vivo models that can accurately predict the efficacy and possible side effects of these agents. As human CMV replication is limited to cells of human origin, there are few animal models available to study ocular or other CMV infections in vivo. Previously, immunosupressed rats (Epstein et al., 1992) and athymic, nude rats (Laycock et al., 1997) have been used as hosts for human retinal tissue transplants. In addition, the use of severe combined immunodeficient (SCID) mice as hosts for human retinal xenografts has been described (Bidanset et al., 1999, 2001; DiLoreto et al., 1994; Kern et al., 2001). In this report, we further describe the use of SCID mice as hosts for human retinal implants (SCID-hu) inoculated with CMV to evaluate the antiviral efficacy of lower concentrations of GCV or CDV on CMV replication as well as to determine if replication rebounds after treatment is terminated.

Human fetal retinal tissue was obtained from Advanced Biosciences Resources, Alameda, CA. The tissue was prepared, implanted, and infected with CMV as described previously (Bidanset et al., 1999). Briefly, four- to eight-week old male SCID mice were anesthetized with ketamine (100 mg/kg) and xylazine (15 mg/kg) and proparacaine–HCl (0.5%) instilled in the eyes. A winged infusion needle containing human fetal retinal tissue was inserted into the anterior chamber of both eyes and approximately 5 μl of tissue injected. Using similar procedures, the mice were again anesthetized four weeks after implantation and 10 μl of the Toledo strain of CMV injected into the anterior chamber containing the implant. Treatment with GCV or CDV was

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Table 1
Effect of ganciclovir on the replication of CMV in SCID-hu retinal implant tissue

Day	Vehicle[a]		45 mg GCV/kg[a]		15 mg GCV/kg[a]	
	Percentage positive (*n*)	pfu/ml[b]	Percentage positive (*n*)	pfu/ml	Percentage positive (*n*)	pfu/ml
4	67 (4/6)	6.3 ± 5.7	0 (0/6)	0 ± 0	50 (3/6)	10 ± 12
7	50 (3/6)	20 ± 29	0 (0/6)	0 ± 0	67 (4/6)	11 ± 11
10	50 (3/6)	43 ± 90	0 (0/6)	0 ± 0	50 (3/6)	14 ± 19
14	50 (3/6)	98 ± 146	33 (2/6)	5.4 ± 10	17 (1/6)	1.3 ± 3.1
21	67 (4/6)	2273 ± 4383	67 (4/6)	52 ± 80	50 (3/6)	119 ± 236
28	83 (5/6)	1538 ± 2624	67 (4/6)[c]	43 ± 43[d]	83 (5/6)	1081 ± 1058
42	50 (2/4)	169 ± 333	100 (4/4)	1084 ± 1315	75 (3/4)	3376 ± 4740

[a] Treatments were initiated 24 h after infection and administered intraperitoneally in 0.1 ml doses twice daily for 14 days followed by once daily for the next 14 days.
[b] Values are expressed as the mean CMV titer ± S.D.
[c] $P < 0.01$ compared with vehicle through day 28 (linear regression).
[d] $P < 0.01$ compared with vehicle through day 28 (non-parametric stratified Wilcoxon Rank Sum).

initiated 24 h later and continued for 28 days, at doses that in earlier studies had proved to be well tolerated.

To monitor CMV replication in retinal implants, 3–6 animals were euthanized at various times after infection. The eyes were removed, homogenized individually in 1.0 ml MEM containing 10% FBS, 2 mM L-glutamine, 200 U/ml penicillin, 50 μg/ml gentamicin and 3 μg/ml fungizone, centrifuged and supernatants frozen at −70 °C until assayed for CMV (Bidanset et al., 1999). Since eyes were homogenized in 1.0 ml of media, and were similar in weight, titers were calculated as pfu/ml homogenate. As data were not normally distributed and there were a number of zero values in several of the data sets, we used a non-parametric stratified Wilcoxon Rank sum test to evaluate differences in virus replication between vehicle and drug-treated mice. Days were used as strata and data collected during the entire 28 days of treatment were used. To compare the rate of infection between two groups throughout the entire 28-day treatment period, a general linear regression model was used with percent positive as the dependent variable and days after infection as the independent variable.

To help validate this model, the effect of GCV on replication of CMV in SCID-hu retinal implants was determined. Four weeks after implantation, retinal tissue implants were infected with 2000 pfu of CMV and animals treated i.p. with either vehicle or 15 or 45 mg of GCV/kg twice daily for the first 14 days and once daily for an additional 14 days. The results summarized in Table 1 (Fig. 1) indicated a significant effect on CMV titers ($P < 0.01$) and on infection rates ($P < 0.01$) in a dose-dependent manner. In mice treated with vehicle, CMV replication increased to peak titers by 21 days and then decreased to near undetectable levels by 42 days. In implants treated with GCV, CMV replication was suppressed until treatment was terminated at 28 days. Thereafter, replication was shown to resume. At 28 days post infection, viral titers from the vehicle treated group were 1538 ± 2624 pfu/ml compared with 43 ± 43 pfu/ml in mice that received 45 mg of GCV/kg and 1081 ± 1058 pfu/ml in those given 15 mg of GCV/kg. Within 14 days of decreasing dosing from twice daily to once daily, the observed viral titers were correspondingly higher. Thus, upon reduction of dose, frequency or termination of treatment, CMV titers began to approach those in vehicle-treated animals. These data suggest that while treatment with GCV is effective in inhibiting CMV replication, the virus is merely suppressed and not cleared from the implant.

To further validate this model, the effect of CDV on CMV replication was determined. Four-week old implants were infected with 2000 pfu of CMV and treated i.p. with either vehicle or 25 or 5 mg of CDV/kg once daily for 28 days. The results presented in Table 2, indicated that in comparison to the vehicle control group, viral titers ($P < 0.001$) and rates of infection ($P < 0.01$) were reduced significantly in implants from animals treated with 25 mg CDV/kg and that the effect on CMV replication was dose-dependent. At 21 days, during the peak of CMV replication, mean viral titers of 56 ± 75 pfu/ml were observed in vehicle-treated mice compared with 0 ± 0 pfu/ml and 25 ± 84 pfu/ml in mice

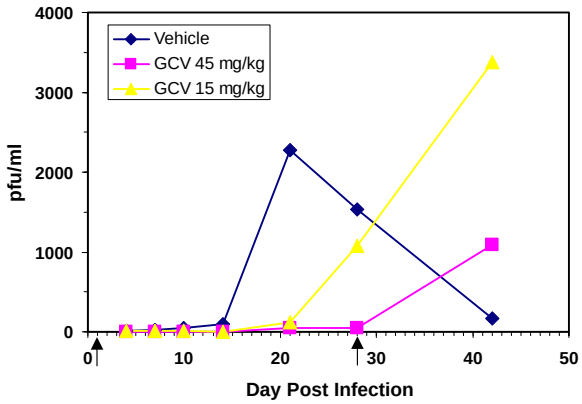

Fig. 1. The effect of ganciclovir (GCV) on the replication of HCMV in SCID-hu retinal tissue implants. Starting 24 h after viral inoculation, SCID mice containing infected retinal tissue implants were treated with vehicle, 45 or 15 mg GCV/kg. At various times after infection, implants were biopsied, homogenized and CMV was quantified by plaque assay. The results compare the effects of treatment with vehicle (◆), 45 mg GCV/kg (■), or 15 mg GCV/kg (▲). Arrows indicate the beginning and end of treatment.

Table 2
Effect of cidofovir on the replication of CMV in SCID-hu retinal implant tissue

Day	Vehicle[a]		25 mg CDV/kg[a]		5 mg CDV/kg[a]	
	Percentage positive (*n*)	pfu/ml[b]	Percentage positive (*n*)	pfu/ml	Percentage positive (*n*)	pfu/ml
7	8 (1/12)	0.6 ± 2.2	8 (1/12)	1.3 ± 4.3	25 (3/12)	3.1 ± 6.8
14	17 (2/12)	5.6 ± 13.2	0 (0/12)	0 ± 0	33 (4/12)	129 ± 432
21	50 (6/12)	55.8 ± 75.0	0 (0/12)	0 ± 0	17 (2/12)	25 ± 84
28	17 (2/12)	7.5 ± 21.7	0 (0/12)[c]	0 ± 0[d]	8 (1/12)	18 ± 63
49	0 (0/14)	0 ± 0	10 (1/10)	1.5 ± 4.7	19 (3/16)	34 ± 111

[a] Treatments were initiated 24 h after infection and administered intraperitoneally in 0.1 ml doses twice daily for 14 days followed by once daily for the next 14 days.
[b] Values are expressed as the mean CMV titer ± S.D.
[c] $P < 0.01$ compared with vehicle through day 28 (linear regression).
[d] $P < 0.001$ compared with vehicle through day 28 (non-parametric stratified Wilcoxon Rank Sum).

treated with 25 or 5 mg CDV/kg, respectively.There are a number of limitations to the use of this model including the availability of human fetal tissue, the tedious nature of implanting, infecting, and harvesting the tissue and the large variation in virus titers from implant to implant and experiment to experiment. In spite of these limitations, the results presented in this paper firmly establishes the value of the SCID-hu retinal implant model for evaluating various antiviral therapies against ocular CMV infections. Standard maintenance therapy for CMV retinitis is costly and often results in side effects including bone marrow suppression, renal insufficiencies, or development of drug resistance (Jabs et al., 2003; Polis, 1999). Thus, it would be useful to have an in vivo model of CMV infection that could predict long-term therapeutic outcome. The results in this study indicate that the antiviral effects of GCV and CDV on CMV replication in SCID-hu retinal tissue implants correlated well with those reported in clinical studies (Jacobson, 1997; The Studies of Ocular Complications of AIDS Research Group in Collaboration with the AIDS Clinical Trials Group, 2001), although the drug concentrations used in the mouse model were somewhat higher than used in humans. In the immunocompromised host (i.e. SCID mouse), it is apparent that although viral replication is inhibited during treatment, the virus is not cleared from the infected xenograft. Upon reduction or termination of treatment, viral replication resumes suggesting that the infection process is only suppressed during the treatment period. Although this model of ocular CMV infection does not mimic the clinical or pathological aspects of CMV retinitis, it does provide a model for CMV infection in which drug is required to penetrate an ocular structure from parenteral administration. The model can provide important information on the course of CMV replication and at least with GCV and CDV is predictive for the virologic outcome.

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