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A model of human cytomegalovirus infection in severe combined immunodeficient mice

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Abstract

Animal models for the evaluation of therapies against human cytomegalovirus (HCMV) are limited due to the species-specific replication of CMV. Models utilizing human fetal tissues implanted into SCID mice have been used but tend to be labor intensive and require human tissues. We therefore developed a model using HCMV-infected human foreskin fibroblasts (HFF) seeded onto a biodegradable gelatin matrix (Gelfoam). Infected HFFs are then implanted subcutaneously into SCID mice. We next evaluated two antivirals in this model. Treatment from days 0 to 5 with ganciclovir (GCV) produced a marginally significant reduction in viral titer while treatment from days 0 to 14 resulted in a more significant reduction in viral titers of 1.47 $\log_{10}$ pfu/ml ($P < 0.0001$). Viral titers were similarly reduced in a group receiving GCV treatment from days 7 to 14 post-implantation (1.50 $\log_{10}$ pfu/ml, $P < 0.0001$). Cidofovir therapy from days 7 to 14 reduced viral titers by almost 2 $\log_{10}$ pfu/ml (from 3.51 ± 0.31 $\log_{10}$ pfu/ml in untreated animals to 1.56 ± 0.40 $\log_{10}$ pfu/ml in treated animals, $P < 0.0001$). These results indicate that the Gelfoam-HCMV SCID mouse model is suitable for the in vivo evaluation of new antivirals against HCMV and is simpler and more convenient than previous models.

Keywords: Human cytomegalovirus (HCMV); SCID mice; Gelfoam; Antiviral; Ganciclovir; Cidofovir

1. Introduction

Although cytomegalovirus (CMV) infections are common, most of the cases in immunocompetent hosts are either asymptomatic or self-limited and require no specific treatment. Infection of the immunocompromised host, however, may result in significant morbidity and mortality even with antiviral therapy (Khare and Sharland, 2001; Pass, 2002). Currently licensed drugs for the treatment of systemic cytomegalovirus infections in the United States include foscarnet, cidofovir and ganciclovir. These compounds are somewhat effective in controlling CMV infections, but emergence of resistance and potentially serious side effects limit their use (Biron, 2006; Boivin et al., 2005; Gilbert and Boivin, 2005). There is also a potential utility for using anti-CMV drugs in patients with congenital CMV infections. Recent data suggests that early treatment of symptomatic congenitally infected infants with intravenous ganciclovir can prevent the progression of hearing loss (Kimberlin et al., 2003). However, safer and more effective orally available compounds are needed to control and improve the outcome of CMV infections.

Animal models of CMV infection utilizing guinea pigs, rats and mice have been very useful in testing new anti-CMV agents (Kern, 2006). However, because of the high species specificity inherent to this virus, testing of new antiviral therapies is restricted to using the specie's own cytomegalovirus. This limitation poses an obstacle for testing of compounds that may show activity only against human cytomegalovirus (HCMV) or with differences in susceptibility between human and animal isolates. One successful approach to overcome this problem has been the use of human tissues such as fetal thymus/liver or retinal tissue surgically implanted in SCID mice and infected with HCMV (Bidanset et al., 2001; DiLoreto et al., 1994; Kern et al., 2001; Mocarski et al., 1993). An alternative approach is to use human tissue culture cells that are placed in a matrix and embedded into mice. For example, hollow fibers containing HCMV-infected human cells have been implanted into SCID mice for antiviral testing (Weber et al., 2001) while Chong et al. (1999) used Gelfoam gelatin sponges as implants. Gelfoam, commonly used for hemostasis in surgical procedures, is a three-dimensional

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matrix with large interstices capable of supporting the growth of cells (Centra et al., 1992). We therefore explored the utility of using these sponges as carriers of HCMV-infected human foreskin fibroblasts and developed a model suitable for evaluation of new anti-HCMV compounds in Gelfoam implanted SCID mice.

2. Material and methods

2.1. Virus and viral cultures

HCMV recombinant virus, HV5.111, a Toledo strain expressing green fluorescent protein (GFP), under the control of the cellular elongation factor 1α (EF1α), was kindly provided by Jeff Vieira (University of Washington, Seattle, WA). The GFP^+ HCMV appears to exhibit growth characteristics similar to wild type virus in cell culture (Jarvis et al., 1999; Iwata et al., 1999; J. Viera, pers. commun.). Since all efficacy comparisons were made with the untreated control implants that were infected with the same GFP^+ virus as the drug treated implants, the differences in behavior and/or properties of the recombinant HV5.111 virus compared to wild type are may not be critical for interpretation of antiviral activity in this model. Viral stocks were grown in human foreskin fibroblasts (HFF, American Type Culture Collection, ATCC CRL 1635, Rockville, MD) that were maintained with DMEM (Invitrogen Corporation, Grand Island, NY) supplemented with 10% fetal bovine serum (Hyclone, Logan, UT) and penicillin/streptomycin 10,000 units/ml (Invitrogen) and stored frozen at −80 °C.

For viral yields, the Gelfoam implants were harvested under sterile conditions from the animal and ground with a glass Dounce homogenizer in 2.0 ml of media. Collagenase type IA (Sigma–Aldrich Corporation, St. Louis, MO) was then added to the media (1.0 mg/ml) to digest the Gelfoam particles present in the sample. The homogenate was then examined by fluorescent microscopy to determine the number of infected cells or sonicated and serially diluted to inoculate HFF monolayers to determine plaque forming units (pfu). For pfu determination, the inoculum was removed after a 2 h incubation period at 37 °C and the monolayers were overlaid with media consisting of 50% Basal Medium Eagle (Sigma–Aldrich Corporation) and 1.5% methylcellulose. The cultures were incubated for 12 days at 37 °C and virus was quantified by counting plaques after staining with crystal violet. The viral CPE was confirmed by immunofluorescence in selected cultures. For statistical analysis, negative cultures were assigned a number corresponding to the limit of detection of the assay ($1.3 \log_{10}$ pfu/ml).

2.2. Mice

C.B.-17 male SCID mice were obtained at 21–28 days of age from Charles River Laboratories (Wilmington, MA). Animals were housed under AAALAC approved facilities and all procedures were approved by the Institutional Animal Care and Use Committee.

2.3. Antivirals

Ganciclovir (GCV) (Roche Laboratories, Nutley, NJ) and cidofovir (CDV) (Gilead Sciences, Foster City, CA) were prepared for animal treatment according to the manufacturer's instructions. GCV was administered twice daily (50 mg/kg dose) by intraperitoneal injection for a total daily dose of 100 mg/kg. Treatment was begun on day 0 or 7 after mice were implanted and continued until day 5 or 14 post-implantation. CDV was administered once daily (25 mg/kg) on days 7–14 post-implantation by intraperitoneal injection.

2.4. Infection and implant procedure

Gelfoam (Cardinal Health, Dublin, OH) was obtained in strips of 2 cm × 6 cm × 7 mm. The strips were aseptically divided into five pieces of ∼4 mm × 6 cm × 7 mm and placed in sterile cryovials. For infection with HCMV, HFFs were first harvested from culture flasks with trypsin, and the cell suspension was centrifuged at 1200 rpm for 10 min. The supernatant was discarded and the cell pellet resuspended in fresh media. The cells were counted using a hemocytometer and infected with HCMV with MOIs ranging from 0.01 to 0.1. The infected cell suspension was placed back in a tissue culture flask containing fresh media and incubated for varying times 37 °C. The following day the infected cells were trypsinized, counted and resuspended in media. Approximately 300 μl of the cell suspension was dispensed into each cryovial containing a Gelfoam strip and again incubated overnight at 37 °C.

For implantation into mice, the Gelfoam strips were loaded into an 11-gauge trocar needle (Popper & Sons, New Hyde Park, NY) using sterile forceps. The mice were anesthetized with sodium pentobarbital and the Gelfoam strips were implanted subcutaneously by entering the area of the upper back and directing the needle towards the lower back down to the dorsal area located just above the hips. One Gelfoam strip was implanted into each mouse in all in vivo experiments.

2.5. Statistics

Means were compared by Student's *t*-test. All comparisons were two-tailed.

3. Results

3.1. In vitro experiments

To determine the optimal growth conditions of the HCMV recombinant virus-infected HFFs on the Gelfoam strips, a series of evaluations were performed in vitro. Factors such as the number of cells used for each strip, the MOI, and the incubation periods were examined. First, the number of cells was determined by seeding the Gelfoam strips with either 1×10^5 or 5×10^5 HFFs after infecting them with HCMV at an MOI of 0.01. Higher viral yields were found with the Gelfoam strips seeded with the higher number of infected cells than in those