Activities of Benzimidazole D- and L-Ribonucleosides in Animal Models of Cytomegalovirus Infections

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Since human cytomegalovirus (HCMV) does not infect or replicate in nonhuman cells and tissues, there are few animal models currently available for evaluation of antiviral therapies for these infections. In the present studies, we utilized two different models in which severe combined immunodeficient (SCID) mice were implanted with human fetal tissue that was subsequently infected with HCMV. In one model, human fetal retinal tissue was implanted into the anterior chamber of the SCID mouse eye, and in the second, human fetal thymus and liver (thy/liv) tissues were implanted under the kidney capsule. After the implants were established, they were infected with 2,000 to 9,000 PFU of HCMV. To determine the efficacy of three benzimidazole nucleosides, 2-bromo-5,6-dichloro-(1-β-D-ribofuranosyl)benzimidazole (BDCRB), GW275175X (175X), and GW257406X (1263W94, maribavir [MBV]) treatment was initiated 24 h after infection of the implants and continued for 28 days. Treatment consisted of either placebo, 25 mg of ganciclovir (GCV)/kg of body weight administered intraperitoneally (i.p.) twice daily, 33 or 100 mg of BDCRB/kg administered i.p. twice daily, or 75 mg of either MBV or 175X/kg administered orally twice daily. GCV was effective in both models, inhibiting HCMV infection by 5- to 3,000-fold. In the retinal tissue model, MBV and BDCRB reduced HCMV replication about fourfold through 21 days postinfection compared with results for the vehicle control. In the thy/liv tissue model, all three benzimidazole nucleosides were effective in inhibiting HCMV replication by approximately 30- to 3,000-fold in comparison to the vehicle control. These data indicate that the benzimidazole nucleosides were efficacious in these animal models and suggest that this class of compounds should be active against the various HCMV infections that occur in the immunocompromised host.

Human cytomegalovirus (HCMV) infections can cause a wide range of clinical manifestations, especially in the immunocompromised or immunosuppressed host. In these patients, a primary HCMV infection or a reactivation of a latent infection can result in an infectious mononucleosis-like syndrome, pneumonia, hepatitis, gastrointestinal disorders, encephalopathies, and particularly in AIDS patients, retinitis. To date, only five antiviral drugs, ganciclovir (GCV), valganciclovir, foscarnet (PFA), cidofovir (CDV), and fomivirsen, have been approved and licensed by the Food and Drug Administration for use in patients with HCMV infection, and all have limitations that preclude their use long term. These limitations include poor oral bioavailability, toxicity, and selection of resistant mutants.

There are a number of previous studies documenting the in vitro activity of the benzimidazole ribonucleosides against HCMV (4, 12, 24, 31, 36, 39). Although the original compounds 2,5,6-trichloro-(1-β-D-ribofuranosyl) benzimidazole and its 2-bromo homolog (BDCRB) were potent and selective inhibitors of HCMV replication in vitro and were bioavailable when delivered orally, they had a short plasma half-life (11). In order to obtain more stable compounds, a number of new analogs were synthesized (32), including the ribopyranosyl analog of BDCRB, termed GW 275175X (175X) (9, 32, 34), and 2-isopropylamino-5,6-dichloro-(1-β-L-ribofuranosyl) benzimidazole (1263W94 or maribavir [MBV]). These compounds had antiviral activity against HCMV that was comparable or better than that of GCV and were active against GCV-or PFA-resistant isolates (4, 36). Additionally, both 175X and MBV were also active against Epstein-Barr virus (36, 38). None of the three nucleoside analogs was active against herpes simplex virus type 1 or 2, varicella-zoster virus, human herpesvirus type 6, or human herpesvirus type 8 (36). The compounds were not active against murine, rat, or guinea pig cytomegalovirus (CMV) strains, which has precluded the evaluation of these compounds in animal models for HCMV infections (36). The pharmacokinetics and toxicity of MBV have been evaluated both in animals and in humans, and good oral bioavailability and low toxicity were reported (18, 35). One clinical trial has been conducted in which a small number of patients with HCMV were given MBV, and a reduction in titers of HCMV in semen was reported (21).

Currently, there are few animal models that can be used to study the biology of HCMV and determine the efficacy of various antiviral therapies. This is largely due to the fact that HCMV infection and replication are limited to human cells. For this reason, the use of immunosuppressed or immunocompromised animals as hosts for human xenografts and later infection of the grafts with HCMV was developed to provide a model for in vivo determination of antiviral drug efficacy (2, 3, 16). In previous studies, we have used severe combined immunodeficient (SCID) mice as hosts for human fetal retinal implants and have been able to successfully show that HCMV...
replicates in the implanted tissue and that GCV and CDV, which are efficacious in the treatment of HCMV infections, are also effective in this model (2, 16).

In addition to retinal tissue, peripheral blood mononuclear cells (PBMC) and retinal cells have been implanted under the kidney capsule of SCID mice and used to examine the replication of HCMV (5, 13). Currently we are also utilizing this model for hosts for human fetal thy/liv tissue implants to determine the efficacy of various antiviral therapies against HCMV replication (16). In this model, thy/liv tissues implanted under the kidney capsule have been shown to fuse and become vascularized. The hematopoietic progenitor cells of the thy/liv implant can then differentiate and proliferate within the environment of the kidney capsule. Like the retinal implants, this tissue can then be infected with HCMV and used to evaluate the efficacy of new potential antiviral therapies for HCMV infection.

The purpose of the studies reported here was to evaluate the effectiveness of therapy with BDCRB, 175X, and MBV with these two animal models for HCMV. Efficacy was compared directly with that obtained with GCV.

MATERIALS AND METHODS

Compounds and reagents. The benzimidazole ribonucleosides, BDCRB, 175X, and MBV, were provided through the Antiviral Research Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health (Bethesda, Md.). CDV and GCV were purchased from the United States Pharmacopeia (Rockville, Md.). L-glutamine, penicillin, and gentamicin were obtained from GIBCO-BRL (Gaithersburg, Md.). Allantoic fluid, FBS, fetal bovine serum, and Eagle’s MEM were obtained from BBL (Becton Dickinson, Cockeysville, Md.). PBS was obtained from Sigma Chemical Co. (St. Louis, Mo.). FGF was obtained from the Collaborative Research Inc. (Bedford, Mass.). All other biochemicals and solvents were of reagent grade and were obtained from standard commercial suppliers.

Tissue, cells, and viruses. Human fetal tissue was obtained from Advanced Biosciences Resources (Alameda, Calif.) and prepared as described below. Human foreskin fibroblast cells were prepared as primary cultures and used in assays with HCMV. These cells were propagated in MEM containing 10% FBS. MBV and BDCRB were prepared as standard virological methods. The Toledo strain of HCMV was obtained from Edward Mocarski (Palo Alto, Calif.).

Plaque reduction assay for HCMV with liquid overlay. Human foreskin fibroblast cells were seeded into six-well plates and incubated at 37°C. Two days later, drug was serially diluted 1:5 in MEM with 2% FBS using six concentrations of drug with a starting concentration of 100 μg/ml. GCV was used as a positive control. The virus to be used was diluted in MEM containing 10% FBS to a desired concentration which gave 20 to 30 plaques per well. Medium was aspirated from the wells, and 0.2 ml of virus suspension was added to each well in triplicate, with 0.2 ml of medium being added to drug toxicity wells. Plates were incubated for 1 h with shaking every 15 min, and drug was added to each well. After incubation for 8 days, the cells were stained with a 5% neutral red solution in phosphate-buffered saline. Stain was aspirated, wells were washed with phosphate-buffered saline, and plaques were counted by using a stereomicroscope. Under these assay conditions, secondary plaque formation did not alter susceptibility levels. By comparing drug-treated wells with untreated controls, 50% effective concentrations were calculated by using MacSynergy II software (27).

SCID-hu mouse model for HCMV replication in ocular tissue. Implantation of human fetal retinal tissue and later infection of these implants were performed as described previously (2). In brief, 4- to 8-week-old male SCID mice were anesthetized with an intraperitoneal (i.p.) injection of ketamine (100 mg/kg of body weight) and xylazine (15 mg/kg), and the topical anesthetic, proparacaine-HCl (0.5%), was instilled in the eyes. A 27- by 1/2-in-gauge winged infusion needle containing mechanically dissociated human fetal retinal tissue was then inserted into the nasal sclera and into the anterior chamber. At the temporal side of the anterior chamber, approximately 5 μl of tissue was injected, and the needle was removed. Using similar procedures, the mice were again anesthetized 6 to 9 weeks after implantation, and 10 μl of 2,000 to 7,500 PFU of HCMV, depending on the experiment, was injected into the anterior chamber containing the implant. Within a given experiment all animals received the same size of inoculum. Beginning 24 h after viral inoculation, the mice were treated with 0.1 ml of drug i.p. or 0.2 ml of drug orally once or twice daily for 28 days, according to the treatment schedules below. On days 7, 14, 21, and 28, four to six animals per group were sacrificed, and their eyes were harvested.

SCID-hu mouse model for HCMV infection in thy/liv tissue. For the second experimental infection, 4- to 6-week-old male SCID mice were anesthetized, and fragments of human fetal thy/liv were implanted under the capsule of one kidney with an 18-gauge trocar, using techniques described previously (5, 13, 25). Following an implant growth period of 12 to 14 weeks, the grafts were inoculated with 2,000 to 9,000 PFU of HCMV, depending on the particular experiment. Beginning 24 h after viral inoculation, the mice were treated daily for 28 days, according to the treatment schedule described below. On days 14, 21, 28, and 35, 5 to 11 implants were biopsied (approximately 50% of the graft size), homogenized, and frozen at −70°C until assayed for HCMV. Biopsies were also obtained 1 week after treatment ended (day 35) to determine if viral replication reappeared after cessation of treatment.

Viral replication in implant tissue. To monitor HCMV replication in retinal implant tissue, animals were sacrificed at various times after infection. The eyes were removed, temporarily stored in sterile irrigating balanced salt solution, and homogenized in MEM containing 10% FBS, 2 mM L-glutamine, 200 U of penicillin/ml, 50 μg of gentamicin/ml, and 3 μg of Fungizone/ml. Eyes were homogenized in 1.0 ml of medium, using a Kontes tissue grinder (Vineland, N.J.) regardless of weight, so titers were calculated as numbers of PFU per milliliter of homogenate. The homogenate was centrifuged at 1,500 rpm for 15 min at 4°C in an Eppendorf 5410R centrifuge, and the supernatant was removed and frozen at −70°C until assayed for HCMV using standard plaque assay techniques. Biopsy samples of thy/liv implants were weighed and homogenized as 10% (wt/vol) suspensions, centrifuged, and assayed as described above. Titers were expressed as log10 PFU/gram of tissue.

Evaluation of efficacy: statistics. In order to determine therapeutic efficacy with these models, animals treated with the benzimidazoles, GCV, or CDV were compared to vehicle-treated animals. Percentages of implants positive for HCMV infection were calculated and compared using a general linear regression model, and titers of virus (no. of PFU/ml ± standard deviation or log10 PFU/g) were compared using a stratified Wilcoxon rank sum test. The results obtained throughout the entire 28-day treatment period were used to calculate significance. In general, a P value of 0.05 or less was considered significant.

RESULTS

In vitro activity against cytomegaloviruses. The activities of the benzimidazole nucleosides have been determined previously for both laboratory and clinical isolates of HCMV as well as for murine, rat, and guinea pig CMVs (36). Results indicated that the low-passage Toledo strain of HCMV used in these studies was inhibited by all three compounds at levels that were equal to or less than those required for other laboratory and clinical isolates, indicating that the Toledo strain is appropriate for use in animal model infections designed to evaluate antiviral activity in vivo.

HCMV is species specific and does not infect experimental animals. Consequently, surrogate animal strains of CMV, such as murine, rat, guinea pig, and to a lesser extent rhesus monkey strains, have been utilized as animal model infections for the study of the biology and development of antiviral drugs directed against CMV infection in humans (1, 28). The activities of BDCRB, MBV, and 175X were compared with those of GCV against human, murine, rat, and guinea pig CMV strains, and these results have been reported previously (36). Although there was appreciable variability in the toxicity of the compounds in primary mouse and guinea pig cells, calculation of a selectivity index indicated that there was little activity of the three compounds against the murine and rat viruses compared with that of GCV, and only BDCRB showed activity against guinea pig CMV.

Effect of treatment with BDCRB on HCMV replication in a SCID-hu mouse ocular model. Since the benzimidazoles are not active against the usual surrogate murine, rat, and guinea...
pig CMV strains used for evaluating efficacy, we have utilized two models of HCMV in SCID mice. In the first experiment, we determined the efficacy in the SCID-hu mouse ocular model. SCID mice with retinal implants infected with the Toledo strain of HCMV were treated i.p. with BDCRB over the course of 28 days as follows. Starting 1 day after infection, mice were infected with 50 mg of GCV/kg in PEG-400 control and the 50-mg/kg BDCRB dose were administered twice daily for 1 week followed by once daily for the last 14 days. Starting 1 day after infection, mice were treated with BDCRB over the course of 28 days. Starting 1 day after infection, mice were sacrificed, eyes were removed and homogenized, and HCMV replication was quantified by plaque assay. These results are shown in Table 1. In comparison with PEG-400-treated animals, treatment with GCV significantly reduced mean titers of virus in implants on day 28 from 1,399 ± 777 PFU/ml in control implants to 15 ± 30 PFU/ml. Implants from mice treated with 50 mg of BDCRB/kg showed a slight but not statistically significant reduction in mean titers of virus, whereas results from animals treated with BDCRB at 25 mg/kg showed no reduction in mean titers of virus. These data suggested that BDCRB was only slightly effective against HCMV at 50 mg/kg and indicated the need to determine the efficacy of BDCRB at higher concentrations.

In a second experiment, SCID-hu retinal implants infected with HCMV were treated i.p. with CDV or BDCRB over the course of 28 days. Starting 1 day after infection, mice were treated twice daily with vehicle (corn oil), 25 mg of CDV/kg, or 75 mg of BDCRB/kg. On days 7, 14, 21, and 28 after infection, mice were sacrificed, eyes were removed and homogenized, and HCMV replication was quantified by plaque assay. These results are also shown in Table 1. In comparison with implants from animals treated with vehicle, treatment with CDV significantly reduced HCMV replication at all time points examined. On day 28, HCMV replication was highest in the vehicle control group, with titers of 774 ± 865 PFU/ml. Replication was reduced significantly (P < 0.0001) in the CDV treatment group but not in implants of animals treated with 75 mg of BDCRB/kg. These data indicated that both GCV and CDV were effective in reducing HCMV replication in retinal implant tissue, but BDCRB did not reduce virus titers significantly.

**Effect of MBV and 175X on HCMV replication in a SCID-hu mouse ocular model.** The short plasma half-life of BDCRB in rodents (7) led to the synthesis of a number of additional analogs, including MBV and 175X. To determine the activities of these compounds, we next utilized SCID mice containing retinal implants infected with the Toledo strain of HCMV. Mice were treated orally with MBV or 175X over the course of 28 days. Starting 1 day after infection, animals were treated either once daily i.p. with 33 mg of GCV/kg in 0.5% methylcellulose or twice daily orally with vehicle (0.5% methylcellulose) and HCMV replication was quantified by plaque assay. The results are shown in Table 2. In comparison to implants from vehicle-treated animals, HCMV replication in implants of animals treated with GCV was significantly reduced at all time points. At 21 days after infection, peak titers of virus in implants from animals treated with vehicle were 656 ± 1,010 PFU/ml. In contrast, titers of virus in implants of animals treated with 33 mg of GCV/kg were approximately threefold lower (228 ± 482 PFU/ml). The overall effect of GCV was significant at P values of <0.01. In addition, HCMV replication in implants from animals treated with either dose of MBV was significantly reduced by approximately threefold. At 75 mg of MBV/kg, titers of virus were reduced to 183 ± 271 PFU/ml, and at 25 mg/kg, titers of virus were 209 ±432 PFU/ml. Although treatment with 175X was effective at 14 days after infection, it appeared that the drug was ineffective at both doses 21 and 28 days after infection. These data indicate that MBV appears to be an excellent candidate for further study, whereas 175X was less effective than MBV or GCV against HCMV replication in the SCID-hu retinal implant model.

**Effect of treatment with BDCRB, MBV, or 175X on HCMV replication in SCID-hu thy/liv tissue implants.** Since infection of the eye with HCMV presents a strong blood-eye barrier to the systemic delivery of an antiviral compound, we next used
SCID mice implanted with human fetal thy/liv tissue under the kidney capsule to determine the efficacy of the benzimidazole ribonucleosides against HCMV replication in a visceral organ. In this model, the implanted thy/liv tissues have been shown to fuse and become vascularized. The hematopoietic progenitor cells of the thy/liv implant can then differentiate and proliferate within the environment of the kidney capsule (25). It might be expected that antiviral drugs that do not penetrate well into the eye may be more efficacious in this model.

The effect of BDCRB was first examined in the thy/liv tissue implant model. In the first experiment, 17-week-old thy/liv implants were infected with 6,700 PFU of the Toledo strain of HCMV. Starting 24 h after infection, mice were treated with vehicle (corn oil), 25 mg of GCV/kg, or 100 or 33 mg of MBV/kg for 28 days. At 14, 21, and 28 days after infection, implants were biopsied and HCMV titers were quantified by plaque assay. The results (Table 3) indicated that both GCV and BDCRB were efficacious in this model. At 28 days after infection, during the peak of HCMV replication, GCV inhibited HCMV replication by greater than 3 log10 PFU/g and both doses of BDCRB inhibited viral replication by approximately 2 to 3 log10 PFU/g.

The effects of the benzimidazole nucleosides, 175X and MBV, on the replication of HCMV were further investigated with the thy/liv tissue implant model. In this experiment, thy/liv implants were infected with 7,500 PFU of the Toledo strain of HCMV. Beginning 24 h after infection, mice were treated with either vehicle (0.5% methylcellulose in saline) or 25 mg of GCV/kg, 100 mg of MBV/kg, or 100 mg of 175X/kg. Treatment was continued for 28 days, and implants were biopsied 14, 21, 28, and 35 days postinfection. Implant tissue was homogenized, and HCMV titers were determined by plaque assay. The results in Table 4 indicated that both 175X and MBV were as effective as GCV in inhibiting the replication of HCMV in thy/liv tissue implants. During benzimidazole nucleoside drug administration, HCMV replication was significantly reduced by 1.5 to 3 log10 PFU/g when compared to the vehicle control.

To complete these studies, an experiment was performed to determine the dose response of treatment with 100 and 33 mg of these two compounds/kg. In this experiment, thy/liv implant tissue was infected with 4,000 PFU of the Toledo strain of HCMV. Beginning 24 h after infection, animals were treated once daily i.p. with 33 mg of GCV/kg or twice daily orally with vehicle (0.5% methylcellulose in saline), 100 mg of MBV/kg, or 100 or 33 mg of 175X/kg. Treatment was continued for 28 days, and implants were biopsied 14, 21, 28, and 35 days postinfection. Implant tissue was homogenized, and HCMV titers were quantified by plaque assays. The results, shown in Table 5, indicate that only treatments with 33 mg of GCV/kg or 100 mg of MBV/kg were effective in inhibiting the replication of HCMV. Based on stratified Wilcoxon rank sum analysis of the data, this inhibition was found to be statistically significant, with P values of 0.0003 for treatment with 33 mg of GCV/kg and 0.0002 for treatment with 100 mg of MBV/kg. At 28 days postinfection, titers of HCMV were 4.97 ± 5.02, 1.22 ± 1.69, 0 ± 0, and 3.72 ± 3.89 log10 PFU/g in implants from animals treated with vehicle, 33 mg of GCV/kg, 100 mg of MBV/kg, and 100 mg of 175X/kg, respectively. Although we observed a 1-log decrease in titer of virus after treatment with 100 mg of 175X/kg, this decrease was not statistically significant. These results suggest that of the three benzimidazole ribonucleoside compounds examined, MBV appears to be the best candidate for further evaluation.

Overall, these experiments with the benzimidazole nucleo-

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**TABLE 2. Effect of treatment with i.p. GCV, oral MBV, or 175X on replication of HCMV in SCID-hu retinal implant tissue**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Positive on day postinfectiona</th>
<th>Titer of virus (no. of PFU/ml) on day postinfectionb</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7</td>
<td>14</td>
<td>21</td>
</tr>
<tr>
<td>Vehicle</td>
<td>33 (4/12)</td>
<td>100 (12/12)</td>
<td>67 (8/12)</td>
</tr>
<tr>
<td>GCV-33</td>
<td>30 (3/10)</td>
<td>58 (7/12)</td>
<td>50 (6/12)</td>
</tr>
<tr>
<td>MBV-75</td>
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<td>MBV-25</td>
<td>9 (1/11)</td>
<td>67 (8/12)</td>
<td>67 (8/12)</td>
</tr>
<tr>
<td>175X-25</td>
<td>25 (3/12)</td>
<td>75 (9/12)</td>
<td>92 (11/12)</td>
</tr>
<tr>
<td>175X-25</td>
<td>8 (1/12)</td>
<td>58 (7/12)</td>
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</tbody>
</table>

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**TABLE 3. Effect of i.p. treatment with GCV or BDCRB on replication of HCMV in SCID-hu thy/liv implant tissue**

<table>
<thead>
<tr>
<th>Treatmenta</th>
<th>% Positive on day postinfectionb</th>
<th>Titer of virus (log10 PFU/g) on day postinfectionc</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>14</td>
<td>21</td>
<td>28</td>
</tr>
<tr>
<td>Vehicle</td>
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<tr>
<td>BDCRB-100</td>
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<td>71 (5/7)</td>
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<tr>
<td>BDCRB-33</td>
<td>56 (5/9)</td>
<td>67 (4/6)</td>
<td>44 (4/9)</td>
</tr>
</tbody>
</table>

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* a Treatments were initiated 24 h after infection and administered i.p. twice daily for 28 days.
  b Values in parentheses are no. positive/no. tested.
  c Values are expressed as means ± standard deviations.
sides indicated that these compounds had significant activity against HCMV replication in vivo both in the retinal tissue implant and in the thy/liv implant model. These compounds appear to be excellent candidates for further development as inhibitors of HCMV replication.

**DISCUSSION**

Although there are a number of licensed drugs for treatment of CMV infection, treatment is far from optimal due to lack of oral activity, bone marrow suppression, or nephrotoxicity. In addition, the emergence of drug-resistant mutants continues to be a problem in the immunocompromised host. One approach to the development of an orally active, less-toxic drug for the treatment of CMV infection has been the synthesis and evaluation of the benzimidazole ribonucleosides (4, 31, 32, 36). However, many of the newer compounds, including the benzimidazole nucleosides, are not active against HCMV replication in vivo both in the retinal tissue implant and in the thy/liv implant model. These compounds appear to be excellent candidates for further development as inhibitors of HCMV replication.

Of the three compounds, only MBV has been extensively evaluated for pharmacokinetic properties and toxicity. These preclinical studies indicated that the compound was about 90% orally bioavailable in the rat and about 50% in monkeys but was poorly distributed in the brain, cerebrospinal fluid, or vitreous humor and was highly protein bound. The results of the toxicology studies indicated a favorable safety profile (18). Based upon the results from the preclinical and toxicology studies and in the absence of an animal model for CMV infection, MBV was evaluated in a phase I safety and pharmacokinetic trial. The results indicated that the drug was rapidly absorbed after oral administration, had few if any severe adverse effects (35), and in a second phase I dose escalation trial with human immunodeficiency virus-infected men reduced titers of HCMV in semen by about 3 log 10 PFU/ml (21).

Animal models for CMV utilizing murine, rat, and guinea pig CMV have played an important role in the development of antiviral drugs for CMV, including GCV, CDV, and PFA (10, 14, 15, 22, 23, 26, 29, 30). However, many of the newer compounds, including the benzimidazole nucleosides, are not ac-

**TABLE 4.** Effect of treatment with i.p. GCV, oral MBV, or 175X on replication of HCMV in SCID-hu thy/liv implant tissue

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Positive on day postinfection</th>
<th>Titer of virus (log_{10} PFU/g), on day postinfection</th>
<th>P value</th>
</tr>
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<td>14</td>
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<td>28</td>
</tr>
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</tr>
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<td>11 (1/9)</td>
<td>22 (2/9)</td>
</tr>
<tr>
<td>175X-75c</td>
<td>22 (2/9)</td>
<td>33 (3/9)</td>
<td>10 (1/10)</td>
</tr>
</tbody>
</table>

* Treatments were initiated 24 h after infection and administered i.p. twice daily for 28 days.
* Treatments were initiated 24 h after infection and administered i.p. twice daily for 28 days.
* Values in parentheses are no. positive/no. tested.
* Values expressed as means ± standard deviations.

**TABLE 5.** Effect of i.p. GCV, oral MBV, or 175X on replication of HCMV in SCID-hu thy/liv implant tissue

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Positive on day postinfection</th>
<th>Titer of virus (log_{10} PFU/g) on day postinfection</th>
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<td>21</td>
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<td>Vehicle</td>
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<td>175X-35c</td>
<td>55 (6/11)</td>
<td>27 (3/11)</td>
<td>64 (7/11)</td>
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</table>

* Treatments were initiated 24 h after infection and administered orally twice daily for 28 days.
* Treatments were initiated 24 h after infection and administered i.p. once daily for 28 days.
* Values in parentheses are no. positive/no. tested.
* Values expressed as means ± standard deviations.
tive against those surrogate viruses (36). To provide animal models for HCMV, we have utilized SCID mice implanted with either human fetal retinal tissue in the eye or thy/liv tissue under the kidney capsule. The implant tissue can be infected with human CMV (8, 25) after a few weeks and used to evaluate the efficacies of new antiviral agents (3, 16). To validate the two models, the efficacies of GCV and CDV were determined in both ocular and thy/liv implants. The results indicated that both models are useful in determining activity against HCMV in vivo and appear to be predictive for efficacy in humans (16).

In the present study, we compared the activities of BDCRB, MBV, and 175X with GCV in both ocular and thy/liv implants. CDV was also used in one retinal study. In the retinal model, GCV given i.p. was very active in significantly reducing viral replication in the implant and reducing infection rates, as was CDV. However, BDCRB did not alter titers of virus, which was not surprising given its short plasma half-life (11). In contrast, oral MBV at either 75 or 25 mg/kg significantly reduced viral replication, although infection rates were not altered. In spite of the information from the preclinical studies, which indicated that MBV was poorly absorbed into the eye and was highly protein bound (18), twice-daily therapy was highly efficacious. In mice treated with 175X, a significant reduction in titers of virus was observed in animals that received 25 mg/kg but was not observed with 75 mg/kg. There is little preclinical information available on this analog to help interpret those results.

In the next series of experiments, the activities of GCV, BDCRB, MBV, and 175X were determined in SCID mice with thy/liv implants under the kidney capsule. As expected, GCV was very effective in this model of infection, reducing HCMV titers by about 3 log_{10} PFU/g. Similar results have been reported by Mocarski and colleagues (25). Although BDCRB was not efficacious in the retinal model, it was highly effective in reducing viral replication in the SCID-hu thy/liv implants. In this model of infection, both MBV and 175X significantly reduced HCMV replication at concentrations of 75 mg/kg twice daily. Lower doses of 33 mg/kg were not effective.

These results support the concept that CMV infection of the visceral organs is more easily treated than those of the eye or the central nervous system. Although GCV and MBV worked in both models, suggesting that MBV may be efficacious in human CMV infection when given orally, it is difficult to make a direct comparison between oral GCV and oral MBV, since GCV is poorly orally bioavailable in humans but relatively highly bioavailable in mice. In general, in our studies with these two models, GCV and CDV have been more effective in treating the thy/liv infection than those in the eye (16). Similar observations were made in these studies for both BDCRB and MBV.

While these two experimental infections do not provide exact models for CMV retinitis and disseminated CMV in transplant patients, they do provide excellent in vivo systems for determining the effects of treatment on the replication of CMV in growing tissue with innervated blood vessels. The efficacy of MBV in these studies supports the clinical observation that treatment of CMV-infected human immunodeficiency virus patients reduced viral replication in semen and further supports the evaluation of this compound in additional clinical studies.

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