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NOTES

In Vitro and In Vivo Inhibition of Murine Gamma Herpesvirus 68 Replication by Selected Antiviral Agents

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We have evaluated the susceptibility of the murine gamma herpesvirus 68 (MHV-68) to a variety of antiviral agents. The acyclic nucleoside phosphonate analogs cidofovir [(*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl) cytosine], (*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)adenine (HPMPA), and adefovir [9-(2-phosphonylmethoxyethyl)adenine] efficiently inhibited the replication of the virus in Vero cells (50% effective concentrations [EC_{50}s], 0.008, 0.06, and 2.2 μg/ml, respectively). Acyclovir, ganciclovir, and brivudin [(*E*)-5-(2-bromovinyl)-2′-deoxyuridine] had equipotent activities (EC_{50}s, 1.5 to 8 μg/ml), whereas foscarnet and penciclovir were less effective (EC_{50}s, 23 and ≥30 μg/ml, respectively). The novel N-7-substituted nucleoside analog S2242 [7-(1,3-dihydroxy-2-propoxymethyl)purine] inhibited MHV-68 replication by 50% at 0.2 μg/ml. The susceptibilities of MHV-68 and Epstein-Barr virus (EBV) to cidofovir, HPMPA, adefovir, and acyclovir were found to be comparable. However, for penciclovir, ganciclovir, brivudin, and S2242, major differences in the sensitivity of MHV-68 and EBV were observed, suggesting that MHV-68 is not always an optimal surrogate for the study of antiviral strategies for EBV. When evaluated with a model for lethal MHV-68 infections in mice with severe combined immunodeficiency, cidofovir proved to be very efficient in protecting against virus-induced mortality (100% survival at 50 days postinfection), whereas acyclovir, brivudin, and adefovir had little or no effect.

Murine gamma herpesvirus 68 (MHV-68) is a murine gamma herpes virus closely related to Epstein-Barr virus (EBV), human herpesvirus 8, and herpesvirus saimiri (HVS) (5, 16). Like EBV and HVS, MHV-68 may induce lymphoproliferative diseases (20). Little is known about the susceptibility of MHV-68 to antiviral agents. Sunil-Chandra et al. (18, 19) have evaluated the anti-MHV-68 activity of acyclovir (ACV), but no comparative study of the activities of different antiviral agents against MHV-68 has been reported so far. To investigate whether MHV-68 may serve as a surrogate for EBV in the development of antiviral strategies, we evaluated the sensitivity of MHV-68 to a variety of antiherpesvirus agents with known anti-EBV activity. Furthermore, knowledge about the susceptibility of MHV-68 to antiviral drugs may provide deeper insight into the mode of replication of this virus.

MHV-68 clone G2.4 was kindly provided by A. A. Nash (Edinburgh, United Kingdom) and was propagated in Vero B cells. Confluent cultures of Vero B cells grown in 96-well microtiter plates were infected with MHV-68 at a multiplicity of infection that yields a 100% cytopathic effect at 7 days postinfection (p.i.). After a 2-h adsorption period, the virus was removed and serial dilutions of the different compounds were added. The cytopathic effect was recorded microscopically at 7 days p.i. The sources of the compounds were as reported earlier (2, 14). The cytostatic effects of the compounds were determined on growing Vero cell cultures. Cells were seeded at a density of 4,000 cells per well and were allowed to stick to the plates for at least 6 h. Then, serial dilutions of the compounds in 10% fetal calf serum were added. After 3 days the cells were trypsinized and the cell numbers were determined with a Coulter counter.

The anti-MHV-68 activities of the selected compounds are presented in Table 1. ACV and ganciclovir (GCV) had about equipotent anti-MHV-68 activities, whereas penciclovir (PCV) was only a weak inhibitor of MHV-68 replication. Brivudin [(*E*)-5-(2-bromovinyl)-2′-deoxyuridine] (BVDU), a molecule that, like ACV, PCV, and GCV, depends on a virus-encoded thymidine kinase (TK) for activation, inhibited MHV-68 replication in the same concentration range as ACV and GCV. The antiviral activity of cidofovir [(*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine; HPMPC] and (*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)adenine (HPMPA), two molecules that do not depend on the viral TK, appeared to be very pronounced: they exhibited 50% effective concentrations (EC_{50}s) of 0.008 to 0.06 μg/ml; this is 50- to 100-fold lower than the EC_{50}s of ACV and GCV. In contrast, the EC_{50}s of cidofovir and HPMPA for inhibition of herpes simplex virus type 1 (HSV-1) and HSV-2 replication in Vero cells are comparable to those of ACV and GCV (13a). Thus, the active (diphosphorylated) metabolites of HPMPC and HPMPA (i.e., HPMPCpp and HPMPApp) may be expected to be potent inhibitors of the MHV-68 DNA polymerization process. Another acyclic nucleoside phosphonate, adefovir [9-(2-phosphonylmethoxyethyl)adenine] (PMEA), which has potent antiretrovirus and antiherpesvirus activities (10), also efficiently inhibited the replication of MHV-68 (EC_{50}, 2.2 ± 0.5 μg/ml). This EC_{50} is comparable to the EC_{50}s of ACV and GCV and is 10-fold lower than the EC_{50} of foscarnet.

We next evaluated the effects of cidofovir, adefovir, brivudin, and ACV in an animal model of lethal MHV-68 infection (Table 2). To mimic the situation in the immunodeficient host, antiviral efficacy was studied in mice with severe combined immunodeficiency (SCID mice). In immunocompetent mice,

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TABLE 1. In vitro activities of selected antiviral agents against MHV-68 replication[a]

Compound	EC_{50} (μg/ml) for MHV-68[b]	IC_{50} (μg/ml)[c]	MTC (μg/ml)[d]	SI[e]	EC_{50} (μg/ml) for EBV[f]
ACV	1.5 ± 0.2	45 ± 25	>100	30	0.4–2.2
PCV	≥30	150 ± 10	>100	≤5	2.3
GCV	8.0 ± 5.1	30 ± 12	>100	3.7	0.02–0.15
Brivudin	2.9 ± 1.4	125 ± 100	>100	43	0.03
Foscarnet	23 ± 11	270 ± 110	>100	11	
S2242	0.2 ± 0.1	21 ± 15	>100	105	0.0007
Cidofovir	0.008 ± 0.003	80 ± 53	>100	10,000	0.01
HPMPA	0.06 ± 0.01	15 ± 7	>100	250	0.02
Adefovir	2.2 ± 0.5	55 ± 35	>100	25	0.3

[a] Data are mean values for three separate experiments.
[b] EC_{50}, 50% antivirally effective concentration, or concentration required to reduce MHV-68-induced cytopathicity in Vero cells by 50%.
[c] IC_{50}, 50% cell growth inhibitory concentration, or concentration required to inhibit the growth of noninfected Vero cells by 50%.
[d] MTC, minimal toxic concentration, or concentration required to alter normal Vero cell morphology.
[e] SI, selectivity index, or IC_{50}/EC_{50} ratio.
[f] Data taken from references 2 and 5 to 8.

suboptimal doses of the antiviral drugs may be sufficient to protect against mortality in the early stages of infection; the mice may mount an efficient immune response and finally survive the infection. In SCID mice, chemotherapeutic means may be the only means of suppressing the infection. In the present study SCID mice were infected intraperitoneally with a dose of MHV-68 that caused 100% mortality in a time span of 2 weeks. At that time, virus was recovered from the lungs, liver, kidney, and spleen (data not shown). Animals were treated subcutaneously for 5 consecutive days a week (until the end of the experiment), starting at 2 h p.i., at a dosage of 25 mg of the different drugs per kg of body weight per day. Neither ACV nor brivudin had any effect on survival in this model, whereas adefovir had some minor effect on the delay in virus-induced mortality. Also, in the study of Sunil-Chandra et al. (19) in which the titers in organs were used as the endpoint for the antiviral activity of ACV, the compound did not cause more than a 10-fold reduction in virus titer. In contrast, we found that cidofovir caused a marked and sustained protection against virus-associated morbidity and mortality. All cidofovir-treated animals survived the infection for more than 50 days p.i. (i.e., for more than 3 weeks after administration of the last dose).

An interesting feature of cidofovir is its prolonged antiviral effect, which lasts even long after the extracellular compound has been removed (11). The long intracellular half-life of the drug metabolites is responsible for this effect (1). The long-lasting antiviral action of cidofovir has also been described in the clinical setting (15). Even after the levels of cidofovir in plasma have fallen well below the EC_{50} for the inhibition of viral replication, the intracellular levels of the drug metabolites are still sufficiently high to inhibit viral replication. This feature of cidofovir may be an important determinant of its marked activity in the MHV-68 infection model in mice. The lack of protection afforded by ACV and brivudin in this model may result from (i) a less efficient intracellular storage of drug metabolites and/or (ii) a lower in vitro susceptibility of the virus to these antiviral agents.

TABLE 2. Inhibitory effects of selected antiviral agents on lethal MHV-68 infection in SCID mice

Treatment (dosage [mg/kg/day])	No. of survivors/ total no. of mice[a]	MDD[b]
Control	0/9	14.4 ± 1.3
Adefovir (25)	2/10[c]	18.7 ± 6.5[c]
ACV (25)	1/10[c]	14.3 ± 0.8[c]
Brivudin (25)	0/10[c]	14.4 ± 2.3[c]
Cidofovir (25)	8/8[d]	>50[d]

[a] At day 50 p.i.
[b] MDD, mean day of death.
[c] Not significant.
[d] $P < 0.001$.

In another experiment, MHV-68-infected SCID mice were treated for 5 consecutive weeks (5 days a week) with 25 mg of cidofovir (subcutaneously) per kg per day. Control animals ($n = 4$) died at 15.7 ± 0.5 days p.i. Cidofovir-treated animals remained healthy for 3 to 4 weeks after therapy was stopped. No virus could be recovered from their organs following cidofovir treatment (as evaluated by titration of homogenized organs [brain, lung, kidney, spleen, and liver] during the 3rd week of treatment). About 1 month after administration of the last dose, signs of MHV-68-associated morbidity started to appear. The animals ($n = 4$) were sacrificed at the time that they were clearly ill (82 ± 5.5 days p.i.). Substantial levels of MHV-68 were detected in the organs of these mice at that time (data not shown). Thus, (i) since viral replication is suppressed during cidofovir treatment but (ii) the animals become ill and ultimately die from the infection about 50 days after the last cidofovir dose has been given, the virus must be reactivated from an inactive state. It has been suggested that MHV-68 persists in latent form in B cells (17). However, since SCID mice do not harbor B cells (4), one must conclude that MHV-68 can also hide in other cells of the organism. Further investigations are needed to determine this site(s) of latency.

MHV-68 has been suggested as a possible surrogate for the study of the pathogenesis and therapy of EBV infections (18, 19). We therefore compared the inhibitory effect of the compounds evaluated in this study with the values for the inhibition of EBV replication reported in the literature (Table 1). The susceptibilities of MHV-68 and EBV to the acyclic nucleoside phosphonates cidofovir, HPMPA, and adefovir, as well as ACV, appeared to be more or less comparable. However, MHV-68 proved to be much less sensitive than EBV to several other compounds, such as GCV, PCV, and S2242 [7-(1,3-dihydroxy-2-propoxymethyl)purine]. This may be related to differences in the metabolism of the compounds in lymphoid cells (in which the anti-EBV assays are performed) compared to that in Vero cells (in which the anti-MHV-68 assays are performed). In fact, we found that S2242 is much more efficiently metabolized in lymphoid cells than in Vero cells (12, 13). In addition, or alternatively, the antiviral agents or their intracellular metabolites may interfere with different potencies with the DNA polymerases of EBV and MHV-68. In conclusion, the study of the susceptibility of MHV-68 to antiviral drugs may provide useful information on the drug sensitivity patterns of gamma herpesviruses. An in vitro infection model in B lymphocytes would eliminate cell line-dependent variations in the intracellular metabolism of the antiviral agents and allow for a direct comparison of the anti-MHV-68 and anti-EBV activities of an antiviral agent in the same cell type.

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