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# Inhibition of Murine Cytomegalovirus Lung Infection and Interstitial Pneumonitis by Acyclovir and 9-(1,3-Dihydroxy-2-Propoxymethyl)Guanine

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**We compared the effects of acyclovir (ACV) and 9-(1,3-dihydroxy-2-propoxymethyl)guanine (DHPG) on murine cytomegalovirus (MCMV) replication in lung and salivary gland tissues, the evolution of interstitial pneumonitis in vivo, and MCMV replication in mouse embryo cells in vitro. As measured by plaque reduction, ACV was more active than DHPG in vitro. In vivo, whether administered orally by gastric intubation or in the drinking water, or subcutaneously, DHPG was more effective than ACV in reducing MCMV titers in lung or salivary gland tissues. This was true in both normal and cyclophosphamide-treated mice. Neither drug was able to prevent MCMV interstitial pneumonitis, despite substantial reductions in virus titer, but both drugs reduced the severity of the pneumonitis.**

Lung infection and interstitial pneumonitis due to cytomegalovirus (CMV) constitute common and often life-threatening problems for individuals whose immune defenses have been altered by disease or medical therapy (3). The pathogenetic mechanisms leading to CMV lung infection and interstitial pneumonitis are poorly understood. However, the advent of successful antiviral chemotherapy for other herpesvirus infections has prompted a search for therapeutic modalities which might improve the prospects of individuals with pulmonary involvement due to CMV. At present, the available chemotherapeutic agents have poor in vitro antiviral activity against most strains of CMV, and uncontrolled therapeutic trials of agents such as interferon, acyclovir (ACV), and vidarabine against CMV interstitial pneumonitis have been disappointing (4, 5, 10, 11). The search for more effective antiviral agents for the treatment of CMV interstitial pneumonitis is an area of intense interest.

We have been studying a model of murine CMV (MCMV) lung infection to better define the roles of host and viral factors in the genesis of MCMV interstitial pneumonitis (8, 9). In BALB/c mice inoculated intranasally with MCMV, there was substantial replication of virus in the lungs without evidence of pneumonitis. However, administration of a single dose of cyclophosphamide (CP) 24 h after virus inoculation induced diffuse interstitial pneumonitis not seen with either virus or CP alone (9). In the studies reported here, we compared the effects of ACV with those of another related antiviral purine analog, 9-(1,3-dihydroxy-2-propoxymethyl)guanine (DHPG), on MCMV replication in lung tissues and on the evolution of interstitial pneumonitis after administration of virus and CP (1, 2, 7, 12). In addition, we compared the antiviral activities of these two compounds on MCMV replication in vitro.

## MATERIALS AND METHODS

**Mice.** For these experiments, 6- to 8-week-old female BALB/c AnN and pregnant CD-1 mice were obtained from Charles River Breeding Laboratories, Inc. (Kingston, N.Y.). Animals were maintained in groups of 5 to 10 in isolator units in accordance with the guidelines of the American Association for Accreditation of Laboratory Animal Care. Animals were permitted unlimited access to Purina Laboratory Chow 500 and water. The colony was monitored for MCMV infection and for other respiratory pathogens.

**Virus.** The Smith strain of MCMV, originally obtained as a 10% (wt/vol) homogenate of infected salivary gland tissue from June E. Osborn (University of Wisconsin, Madison), was used for these experiments. The virus inoculum, prepared from tissue culture supernatants after a single passage in mouse embryo cells, contained $2.0 \times 10^6$ PFU of virus per ml. The inoculum for sham-infected controls was prepared by heating the MCMV pool to 56°C for 60 min. Virus and control stocks were stored at −70°C. The virus stocks were screened and found to be free of other murine pathogens (8).

**Cell culture methods.** Mouse embryo cells were prepared from embryos of late-term pregnant CD-1 mice as previously described (8, 9). Quantitation of virus was performed by plaque assay in mouse embryo cell monolayers under a 1% tragacanth overlay (6, 9).

**Intranasal inoculation of virus.** Animals under light ether anesthesia were inoculated intranasally with $10^5$ PFU of MCMV. Control animals were inoculated in parallel with an equivalent volume of heat-inactivated virus (6, 7).

**Induction of interstitial pneumonitis.** To induce interstitial pneumonitis in the MCMV-infected animals, CP (Sigma Chemical Co., St. Louis, Mo.) was prepared fresh as a 20-mg/ml solution and given as a single dose (0.20 mg/g of body weight) intraperitoneally 24 h after virus inoculation. This procedure, in conjunction with MCMV inoculation, induced histologic evidence of interstitial pneumonitis in >90% of BALB/c mice, whereas pneumonitis was rarely induced in sham-inoculated controls (8, 9).

**Determination of antibody to MCMV.** Titers of serum antibody to MCMV were determined by an enzyme-linked immunosorbent assay as previously described (9).

**Evaluation of virus infection and interstitial pneumonitis.** Mice were killed at various intervals after inoculation, and their body and right lung weights were determined. The lung index, the ratio of the wet weight of the right lung (milligrams) to the body weight (grams), was calculated.

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TABLE 1. Oral ACV and DHPG inhibit MCMV replication in lungs and salivary glands of BALB/c mice

| Drug | Dose | MCMV[a] in: | | | |
|---|---|---|---|---|---|
| | | Salivary gland | | Lung | |
| | | Titer | % Reduction | Titer | % Reduction |
| None | | 6.23 | | 4.64 | |
| ACV (IG[b]) | 50[b] | 5.49 | 83 | 4.48 | 30 |
| | 500 | 4.20 | 99 | 3.28 | 97 |
| ACV | 0.5 (100)[c] | 5.66 | 73 | 4.08 | 72 |
| | 1.5 (300) | 4.84 | 96 | 3.64 | 90 |
| DHPG | 0.2 (40)[c] | 5.43 | 84 | 3.11 | 97 |
| | 0.5 (100) | 4.72 | 97 | 3.00 | 98 |
| | 1.0 (200) | 4.08 | 99 | 2.78 | 99 |
| | 1.5 (300) | 3.23 | 99 | 2.36 | 99 |

[a] MCMV titers ($log_{10}$ PFU per milliliter) were determined from 10% tissue homogenates pooled from five mice per sample 10 days after intranasal inoculation with $10^5$ PFU of virus. The results are compiled from three experiments.

[b] Total daily dose of antiviral drug (milligrams per kilogram of body weight) given by gastric intubation (IG) in two doses per day.

[c] Concentration of antiviral drug (milligrams per milliliter) given ad libitum in the drinking water. The estimated oral dose (milligrams per kilogram of body weight per day) is shown in parentheses.

Homogenates of salivary gland and lung tissues (10% wt/vol) were prepared in minimal essential medium (10% newborn calf serum, 10% dimethyl sulfoxide) and were stored at −70°C until virus assay. Tissues for histologic evaluation were prepared as previously described (7, 9). The samples were coded before examination.

**Antiviral drug administration.** ACV [9-(2-hydroxyethoxymethyl)guanine] was purchased from Burroughs Wellcome Co. (Research Triangle Park, N.C.). DHPG was kindly donated by Syntex Laboratories, Inc. (Palo Alto, Calif.). For subcutaneous (s.c.) administration, fresh stock solutions of each drug were prepared at 2.0 mg/ml with sterile distilled water. The pH was adjusted to 7.5 with 1 M HCl. To vary the drug concentration, the drugs were further diluted in water and were administered s.c. every 12 h beginning 24 h after virus inoculation.

For oral administration, stock solutions of 1.5 mg of drug per ml of distilled water were prepared and diluted appropriately. These dilutions were substituted for drinking water 24 h after virus inoculation (orally). Water intake was monitored, and fresh drug was supplied daily. For several experiments, ACV was given by gastric intubation. A stock solution (50 mg/ml) was prepared and diluted with sterile water; 0.1 ml of the appropriate dilutions was administered at 12-h intervals.

**In vitro assay of antiviral activity.** The antiviral activity of the drugs against MCMV was determined by a plaque reduction assay in mouse embryo cells. Confluent monolayers were infected with approximately 50 PFU of virus. After adsorption for 1 h at 37°C, 2.0 ml of overlay with various dilutions of antiviral drug or no drug was added. All dilutions of antiviral drugs were tested in triplicate. To determine whether the overlay altered the drug effect, three overlays were tested. These included minimal essential medium containing 20% newborn calf serum, 0.02 M HEPES *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) (pH 7.4), penicillin (100 U/ml), gentamicin (50 μg/ml), and 1% gum tragacanth, 0.5% carboxymethyl cellulose, or 0.2% agarose. After incubation at 37°C for 4 days, the monolayers were fixed with 95% ethanol–glacial acetic acid–37% Formalin (6:2:1, vol/vol) and stained with 1% crystal violet, and the number of plaques per well was counted. The antiviral dose that gave 50% plaque reduction was determined.

## RESULTS

**Susceptibility of MCMV to antiviral drugs in vitro.** The in vitro susceptibility of MCMV to ACV was compared with DHPG by using a 50% plaque reduction assay with a 1% tragacanth, 0.5% carboxymethyl cellulose, or 0.2% agarose overlay. The concentrations of ACV and DHPG causing 50% inhibition of MCMV plaque formation were 0.85 and 3.8 μg/ml, respectively, regardless of the overlay.

**Inhibition of MCMV lung infection in vivo.** Previous work demonstrated that virus replication peak virus titers were attained 6 to 10 days after intranasal inoculation (9). Therefore, we compared the effects of various doses of ACV and DHPG given orally on MCMV replication 10 days after inoculation (Table 1). Mice drank an average of 4 ml of water daily; this consumption was not altered by the addition of drug to the water. At comparable doses, DHPG was more effective than ACV in inhibiting replication of MCMV in both the lungs and salivary glands. Increasing doses of both ACV and DHPG produced progressive inhibition of MCMV replication. ACV was effective in inhibiting MCMV replication when given either orally or by intragastric administration.

Similarly, parenteral administration of both drugs produced a dose-related inhibition of MCMV replication (Table 2). Again, at comparable doses, DHPG was more effective in inhibiting MCMV replication in both the lungs and salivary glands.

**Modification of interstitial pneumonitis.** Previous work has demonstrated that the majority of BALB/c mice develop diffuse interstitial pneumonitis 2 weeks after receiving both MCMV and a single dose of CP. Furthermore, once established, the severity of this pneumonitis is directly related to the virus content of the lungs and can be modified by administration of antiviral drugs (8). Therefore, we compared the abilities of ACV and DHPG to prevent or modify interstitial pneumonitis.

Oral administration of ACV and DHPG 1 day after virus

TABLE 2. s.c. ACV and DHPG inhibit MCMV replication in tissues of BALB/c mice

| Drug | Dose[b] | MCMV[a] in: | | | |
|---|---|---|---|---|---|
| | | Salivary gland | | Lung | |
| | | Titer | % Reduction | Titer | % Reduction |
| None | | 5.59 | | 3.86 | |
| ACV (s.c.) | 5 | 5.65 | 0 | 4.26 | 0 |
| | 50 | 4.84 | 82 | 2.94 | 88 |
| DHPG (s.c.) | 5 | 4.11 | 97 | 3.00 | 86 |
| | 10 | 3.83 | 98 | 2.67 | 94 |
| | 50 | 1.83 | 99 | 2.48 | 96 |

[a] MCMV titers ($log_{10}$ PFU per milliliter) were determined from 10% tissue homogenates pooled from five mice per sample 10 days after intranasal inoculation with $10^5$ PFU of virus.

[b] Total daily dose of antiviral drug (milligrams per kilogram of body weight) given s.c. in two daily doses beginning 24 h after virus inoculation.

TABLE 3. Effects of oral ACV or DHPG on occurrence and severity of MCMV interstitial pneumonitis in BALB/c mice

| Group[b] | Drug treatment (day)[c] | MCMV titer[a] | | Lung index[d] | Interstitial pneumonitis[e] |
|---|---|---|---|---|---|
| | | Salivary gland | Lung | | |
| Sham-CP | None | | | 6.2 ± 0.3 | 0/5 |
| Sham-CP | ACV (1) | | | 5.6 ± 0.6 | ND[f] |
| MCMV-CP | None | 6.58 | 4.60 | 9.4 ± 2.0 | 6/7 |
| MCMV-CP | ACV (1) | 5.40 | 4.01 | 7.8 ± 1.0 | 7/7 |
| MCMV-CP | ACV (7) | 5.95 | 4.29 | 7.7 ± 1.0 | 4/5 |
| MCMV-CP | DHPG (1) | 5.22 | 2.95 | 7.3 ± 0.7 | 6/6 |

[a] MCMV titers ($log_{10}$ PFU per milliliter) were determined from 10% tissue homogenates pooled from at least five mice 14 days after intranasal inoculation with $10^5$ PFU of virus.
[b] Mice were given CP (200 mg/kg of body weight) 24 h after either sham or virus inoculation.
[c] Antiviral drugs (1.5 mg/ml) were provided in the drinking water ad libitum beginning either 1 or 7 days after virus inoculation. The estimated dose was 300 mg/kg of body weight per day.
[d] Mean ± standard deviation of the wet weight of the right lung (milligrams) per body weight (grams).
[e] Number of mice with histologic evidence of interstitial pneumonitis per total at risk.
[f] ND, Not done.

inoculation reduced MCMV replication in both lungs and salivary glands (Table 3). Although neither drug could prevent histologic development of interstitial pneumonitis, both agents reduced its severity as judged by a decrease in lung weights and lung index and by direct histologic examination. For ACV, this effect was seen if antiviral therapy was started as late as 7 days after virus inoculation.

Administration of both ACV and DHPG s.c. also inhibited MCMV replication (Table 4). In this situation, DHPG was substantially more effective in reducing virus replication. Again, however, neither agent was able to prevent interstitial pneumonitis, but both drugs appeared to modify its severity.

## DISCUSSION

These studies demonstrated that both ACV and DHPG inhibited MCMV replication in tissue culture and in both lungs and salivary glands in vivo. Although ACV was more active in vitro, DHPG more effectively inhibited MCMV replication in vivo. Neither drug could prevent the development of MCMV interstitial pneumonitis, but both agents appeared to lessen its severity.

Our understanding of the pathogenesis of MCMV interstitial pneumonitis is incomplete. The contributions to the disease process by direct viral damage or host immune response processes are not defined. Previous work in our laboratory has shown that MCMV alone is insufficient to induce interstitial pneumonitis, despite substantial virus replication in the lungs (8, 9). However, once induced by MCMV and CP administration, the severity of the interstitial pneumonitis is directly related to the virus content of the lungs (8). It was reasonable to expect, therefore, that antiviral therapy which reduced virus replication in the lungs would prevent or at least modify the interstitial pneumonitis. Both ACV and DHPG were able to inhibit lung virus replication, reducing virus titers from 50 to 95%, depending on the conditions of administration. Although neither drug was able to prevent interstitial pneumonitis, both drugs appeared to reduce its severity. It is not clear whether the persistence of interstitial pneumonitis in this setting was due to a failure to treat a host-mediated component of this process or simply to insufficient inhibition of virus replication. Despite a significant reduction in virus content during antiviral treatment, substantial quantities of virus persisted in the lungs. Presumably, additional reduction of virus content might further modify interstitial pneumonitis. Nevertheless, these studies suggest that antiviral modification of MCMV interstitial pneumonitis appears possible. The use of antiviral agents in treatment of human CMV interstitial pneumonitis merits further study.

TABLE 4. Effects of s.c. ACV or DHPG on occurrence and severity of MCMV interstitial pneumonitis in BALB/c mice 14 days after infection

| Group[b] | Drug treatment[c] | MCMV titer[a] | | Lung index[d] | Interstitial pneumonitis[e] |
|---|---|---|---|---|---|
| | | Salivary gland | Lung | | |
| Sham-CP | None | | | 5.8 ± 0.6 | 2/9 |
| Sham-CP | ACV | | | 6.6 ± 0.7 | |
| Sham-CP | DHPG | | | 5.8 ± 1.0 | |
| MCMV-CP | None | 6.80 | 5.04 | 10.3 ± 0.2 | 8/9 |
| MCMV-CP | ACV | 5.52 | 4.08 | 7.6 ± 1.0 | 12/13 |
| MCMV-CP | DHPG | 2.52 | 2.48 | 7.8 ± 0.9 | 10/11 |

[a] MCMV titers ($log_{10}$ PFU per milliliter) were determined from 10% tissue homogenates pooled from at least five mice per sample 14 days after intranasal inoculation with $10^5$ PFU of virus.
[b] Mice were given CP (200 mg/kg of body weight) 24 h after either sham or virus inoculation.
[c] Antiviral drugs (50 mg/kg of body weight per day) were given s.c. in two doses beginning 1 day after virus or sham inoculation.
[d] Mean ± standard deviation of the wet weight of the right lung (milligrams) per body weight (grams).
[e] Number of mice with histologic evidence of interstitial penumonitis per total at risk.

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