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Activity and Mechanism of Action of *N*-Methanocarbathymidine against Herpesvirus and Orthopoxvirus Infections

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***N*-Methanocarbathymidine [(*N*)-MCT] is a conformationally locked nucleoside analog that is active against some herpesviruses and orthopoxviruses in vitro. The antiviral activity of this molecule is dependent on the type I thymidine kinase (TK) in herpes simplex virus and also appears to be dependent on the type II TK expressed by cowpox and vaccinia viruses, suggesting that it is a substrate for both of these divergent forms of the enzyme. The drug is also a good inhibitor of viral DNA synthesis in both viruses and is consistent with inhibition of the viral DNA polymerase once it is activated by the viral TK homologs. This mechanism of action explains the rather unusual spectrum of activity, which is limited to orthopoxviruses, alphaherpesviruses, and Epstein-Barr virus, since these viruses express molecules with TK activity that can phosphorylate and thus activate the drug. The compound is also effective in vivo and reduces the mortality of mice infected with orthopoxviruses, as well as those infected with herpes simplex virus type 1 when treatment is initiated 24 h after infection. These results indicate that (*N*)-MCT is active in vitro and in vivo, and its mechanism of action suggests that the molecule may be an effective therapeutic for orthopoxvirus and herpesvirus infections, thus warranting further development.**

The most commonly used therapies for herpesvirus infections derive their specificity primarily through selective phosphorylation by viral kinases. Acyclovir (ACV) and penciclovir are phosphorylated by the thymidine kinases (TK) encoded by herpes simplex virus (HSV) and varicella-zoster virus, and the active triphosphate metabolites are potent inhibitors of the viral DNA polymerases (3, 5, 12, 13). These compounds are ineffective against betaherpesviruses since they do not encode TK homologs, yet a closely related nucleoside, ganciclovir (GCV), is specifically phosphorylated by the human cytomegalovirus UL97 kinase (21, 39). Indeed, this strategy could be utilized against any virus provided that (i) a nucleoside could be identified that is a substrate for a viral kinase and (ii) the phosphorylated metabolite is an inhibitor of the viral polymerase. Orthopoxviruses are ideal candidates for such an approach since they encode highly conserved DNA polymerases and they fortuitously express enzymes with TK activity (17). The type II TK homologs in orthopoxviruses differ significantly from the type I homologs encoded by herpesviruses in several respects, including molecular weight, quaternary structure, and a more limited substrate specificity (4). ACV, GCV, and penciclovir are not substrates for this enzyme, and as a consequence, the drugs are inactive against orthopoxviruses. In order to identify new antiviral agents for the treatment of orthopoxvirus infections, we sought to identify nucleoside analogs that were dependent on the cowpox virus (CV) TK for their antiviral activity and, by inference, should be selective inhibitors of this virus and other viruses that express enzymes with TK activity. Herein, we describe the antiviral activity of (*N*)-methanocarbathymidine [(*N*)-MCT], which is active against orthopoxviruses and some herpesviruses and is specifically activated by the distinct TK homologs expressed by these diverse virus families.

New therapies are required not only to treat potential infections by monkeypox virus and variola virus but also to treat adverse events related to vaccination with vaccinia virus (VV) (6, 7, 14). A number of compounds have been described that inhibit replication of orthopoxvirus at numerous points in its life cycle (28), and their antiviral activity has been reviewed elsewhere (10). Cidofovir (CDV) is approved for the therapy of cytomegalovirus retinitis in patients with AIDS and is under an investigational new drug application for the emergency treatment of smallpox and complications from vaccination. CDV has been shown to possess good antiviral activity against a wide spectrum of viruses including VV, CV, variola virus, ectromelia virus, and monkeypox virus (2, 11, 18, 24). There are also anecdotal reports of successful CDV treatment for molluscum contagiosum and orf virus infections (9, 15). The usefulness of this drug, however, is limited by a lack of oral bioavailability and toxicity (8). New orally bioavailable analogs of CDV with greatly improved potency are now being developed as potential therapies for the treatment of smallpox infections (19, 31), and other compounds with good activity in animal models have been identified (25, 35–37). Results presented here add (*N*)-MCT to the few compounds with good in vivo activity.

The synthesis and antiviral activity of nucleosides containing the (*N*)-methanocarba group in place of deoxyribose have been described previously (23) (Fig. 1). The thymidine analog (*N*)-MCT containing this sugar moiety exhibited good antiviral activity against herpes simplex virus type 1 (HSV-1), HSV-2, and varicella-zoster virus, and the 5-bromo derivative was shown to have good activity against VV and CV (23, 32). This compound was shown to be selectively phosphorylated by the HSV TK (34), and high levels of the triphosphate metabolite were formed in infected cells (22). The phosphorylation of this

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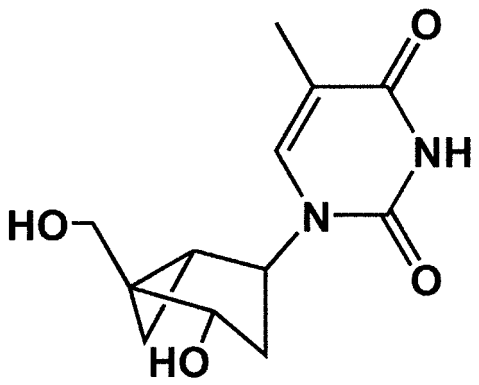

FIG. 1. Structure of (*N*)-MCT.

compound in HSV-1-infected cells is different from that for ACV and GCV in that the HSV-1 TK catalyzes the conversion of (*N*)-MCT monophosphate to (*N*)-MCT diphosphate, whereas this same enzyme catalyzes the formation of ACV and GCV monophosphate from the nucleoside (40). This compound has a thousandfold-greater affinity for the HSV TK than the human homolog does and as a result is highly selective for virus-infected cells (30). Similar studies have not been reported for orthopoxviruses and the phosphorylated metabolites of (*N*)-MCT in VV-infected cells are unknown.

Previously published data that were generated in our laboratory and published elsewhere (32) initially reported the antiviral activity of (*N*)-MCT against HSV-1 and HSV-2 and the activity of the 5-bromo analog against VV and CV. In this report we have confirmed the antiviral activity of (*N*)-MCT against the orthopoxviruses and investigated the function of TK in the mechanism of action of this compound. These studies suggested that this molecule was specifically phosphorylated by the TK homologs in both CV and HSV-1 and that the active metabolite inhibited viral replication at the level of viral DNA synthesis. These data taken together with the pharmacokinetic properties of the drug (26) suggested that it might exhibit antiviral activity in vivo. In the present studies, (*N*)-MCT was tested in a murine model against VV, CV, and HSV-1. Mice treated intraperitoneally (i.p.) with (*N*)-MCT beginning 24 h after infection were protected from lethality with each of these viruses at relatively low doses of drug. Results presented here describe a molecule that can be activated by the divergent TK homologs in orthopoxviruses and some herpesviruses and support the further development of this drug as a possible therapy for these infections in humans.

MATERIALS AND METHODS

Cells and viruses. Methods for producing and passaging human foreskin fibroblast (HFF) cells were described previously (33). Culture medium for all cell lines was minimal essential medium (MEM) containing 10% fetal bovine serum (FBS) and standard concentrations of L-glutamine, penicillin, and gentamicin. VV strains WR, Copenhagen, and IHD were obtained from the American Type Culture Collection (ATCC; Manassas, Va.). Working stocks of these viruses were propagated in Vero cells obtained from the ATCC. CV, strain Brighton, was kindly provided by John W. Huggins (Department of Viral Therapeutics, Virology Division, U.S. Army Medical Research Institute of Infectious Disease, Frederick, Md.). Δ*crmA* (TK^+) and TK:GFP *lacZ* (TK^-) CV strains were obtained from Pete Turner (University of Florida, Gainesville, FL) and were described previously (1). The wild-type HSV-1 strain F and TK^- strain DM2.1 were described and propagated as reported previously (16). CDV (Vistide) was a gift of Gilead Pharmaceuticals (Foster City, CA), and (*N*)-MCT and the other compounds were obtained through the NIAID, NIH, Bethesda, MD.

VV, CV, and HSV plaque reduction assays. For VV and CV, HFF cells were added to six-well plates and incubated for 2 days at 37°C with 5% CO_2 and 90% humidity. On the day of assay, drug at two times the final desired concentration was diluted serially 1:5 in 2× MEM with 10% FBS to provide six concentrations. Aspiration of culture medium from triplicate wells for each drug concentration was followed by addition of 0.2 ml per well of diluted virus, which would give 20 to 30 plaques per well in MEM containing 10% FBS or 0.2 ml medium for drug toxicity wells. The plates were incubated for 1 h with shaking every 15 min. An equal amount of 1% agarose was added to an equal volume of each drug dilution, and this mixture was added to each well in 2-ml volumes and the plates were incubated for 3 days. The cells were stained with a solution of neutral red in phosphate-buffered saline (PBS) and incubated for 5 to 6 h. The stain was aspirated, plaques were counted using a stereomicroscope at 10× magnification, and 50% effective concentrations (EC_{50}s) were calculated by standard methods. The HSV plaque reductions were essentially the same as those for VV and CV with the following changes. The drug solutions were prepared at the desired concentration in MEM with 2% FBS, and a liquid overlay with pooled human serum containing antibodies to HSV instead of agarose was used. At 72 h following infection, the medium containing the drug was aspirated and the monolayers were stained with 1 ml of a solution of 0.01% crystal violet in 60% methanol for 10 min. Residual stain was then washed from the wells with 1 ml PBS, and plaques were counted.

CV β-galactosidase assay. Monolayers of HFF cells in 96-well plates were incubated at 37°C for 24 h in a humidified incubator. Drugs were then diluted in the plates, and either TK^+ or TK^- strains of CV were added at a multiplicity of infection of 0.05 PFU/cell (27). At 48 h postinfection, the medium was removed and the β-galactosidase substrate chlorophenol red–β-galactopyranoside was added at a final concentration of 50 μg/ml in PBS. The conversion of the colorimetric substrate was determined by measuring the absorbance at 570 nm, and EC_{50}s were calculated by standard methods (29). The EC_{50} ratio for TK^- and TK^+ viruses was calculated and used as a measure of TK dependence.

Cytotoxicity determination. The neutral red uptake assay was conducted as reported previously (18). Briefly, HFF cells were plated into 96-well plates at a concentration of 2.5×10^4 cells per well. After 24 h, the medium was aspirated and 125 μl of each drug concentration in MEM with 2% FBS was added to the first row of wells in triplicate. Serial 1:5 dilutions were performed using the Beckman BioMek liquid handling system. After compound addition, the plates were incubated for 7 days in a CO_2 incubator at 37°C. After incubation, the medium/drug was aspirated and 200 μl/well of 0.01% neutral red in PBS was added and incubated for 1 h. The dye was aspirated, and the cells were washed with PBS using a Nunc plate washer. After the PBS was removed, 200 μl/well of a solution containing 50% ethanol and 1% glacial acetic acid was added. The plates were placed on a rotary shaker for 15 min, and the optical densities were determined at 540 nm. The concentration of drug that reduced cell viability by 50% was then calculated. In the proliferation assay, cells were seeded in six-well plates at a concentration of 2.5×10^4 cells/ml. After 24 h, the medium was aspirated and drug serially diluted 1:5 was added to the appropriate wells. The cells were incubated for 72 h at 37°C and then trypsinized and counted using a Coulter counter. Standard methods were used to determine the drug concentration which inhibited cell proliferation by 50%.

DNA synthesis assay. Drugs were diluted to final concentrations of 30, 10, 3, 1, and 0.3 μg/ml and added to confluent monolayers of HFF cells in six-well plates. The WR strain of VV was used to infect the cells at a multiplicity of infection of 0.5 PFU/cell. Total DNA was extracted 24 h postinfection by lysing the cells in a buffer containing 10 mM Tris, pH 7.4, 1 mM EDTA, and 1% sodium dodecyl sulfate and incubating them with 100 μg of proteinase K for 60 min at 37°C. DNA samples were purified using QIAGEN's QiaQuick PCR purification system according to the manufacturer's protocol. Samples were cleaved with EcoRV, and fragments were separated on an agarose gel, transferred to a nylon membrane, and hybridized to a digoxigenin-labeled probe (Roche Applied Science, Indianapolis, IN), specific for VV DNA (coordinates 128257 to 129537 in AY243312).

Activity of (*N*)-MCT in mice inoculated with HSV, VV, or CV. Female BALB/c mice, 3 to 4 weeks of age, were obtained from Charles River Laboratories (Raleigh, N.C.). Mice were group housed in microisolator cages, and each treatment group contained 15 mice. Mice were obtained, housed, utilized, and euthanized according to policies of the USDA and AAALAC. All animal procedures were approved by the University of Alabama at Birmingham Institutional Animal Care and Use Committee prior to initiation of studies. Infections were initiated by intranasal (i.n.) inoculation of BALB/c mice. Mice were infected with an approximate 90% lethal dose of HSV-1 strain E377 (1.0×10^5 PFU/animal),

TABLE 1. Efficacy and cytotoxicity of (*N*)-MCT in HFF cells infected with VV or CV

Drug	VV (Copenhagen)		CV (Brighton)		CC_{50}[a]	IC_{50}[b]
	EC_{50}[c]	SI[d]	EC_{50}[c]	SI[d]		
(*N*)-MCT	0.55 ± 0.13	>182	1.5 ± 1.2	>67	>100 ± 0	29 ± 5.4
CDV	3.3 ± 0.3	>30	4.4 ± 0.6	>23	>100 ± 0	20 ± 7

[a] CC_{50}, 50% cellular cytotoxicity determined by neutral red uptake in stationary monolayers of HFF cells.
[b] IC_{50}, 50% inhibition of cell proliferation.
[c] Average of two or more assays shown in units of μg/ml with standard deviations.
[d] SI, selective index calculated as the CC_{50} divided by the EC_{50}.

TABLE 2. (*N*)-MCT is dependent on orthopoxvirus and herpes simplex virus thymidine kinase homologs for its antiviral activity

Drug	EC_{50} (μg/ml)					
	CV (Δ*crm lacZ*) TK^+	CV (TK:*gfp lacZ*) TK^-	Ratio[b]	HSV-1 (F) TK^+	HSV-1 (DM2.1) TK^-	Ratio[b]
(*N*)-MCT	2.0 ± 2.0[a]	28 ± 2.3	14	0.07 ± 0.02	6.3 ± 3.9	90
CDV	3.5 ± 3.3	4.2 ± 3.9	1.2	1.5 ± 1.1	1.5 ± 1.4	1.0
ACV	NA[c]	NA	NA	0.3 ± 0	>100 ± 0	333
IDU	0.4 ± 0.18	22 ± 9.3	55	2.1 ± 0.1	54 ± 8.1	26

[a] Average of three or more experiments with standard deviation.
[b] EC_{50} ratio for TK^- and TK^+ viruses, respectively.
[c] NA, not applicable.

CV strain BR (3.3×10^4 PFU/animal), VV strain WR (1.0×10^4 PFU/animal), or VV strain IHD (2.5×10^4 PFU/animal) using a micropipettor and a total volume of 40 μl per animal.

(*N*)-MCT was suspended in 0.4% carboxymethyl cellulose (CMC) to yield 50-, 16.7-, or 5.6-mg/kg-of-body-weight doses in a volume of 0.1 ml. Mice were treated i.p. with (*N*)-MCT twice daily, approximately 12 h apart beginning 24 h post-viral inoculation with a 5-day duration of therapy for orthopoxviruses and a 7-day duration for HSV-1. Vehicle-treated mice were included as negative controls, and either ACV or CDV was included as a positive control. Mortality rates were analyzed by Fisher's exact test, and mean day of death was analyzed by Mann-Whitney U rank sum. A *P* value of 0.05 or less was considered significant.

RESULTS

Studies reported here were conducted to further characterize the antiviral activity of (*N*)-MCT and evaluate its potential as a therapy for orthopoxvirus and HSV infections. The antiviral activity of (*N*)-MCT was determined against the Copenhagen strain of VV and the Brighton strain of CV (Table 1). In these standard plaque assays, the drug yielded EC_{50}s against both viruses that were lower than those for the CDV control. Cytotoxicity was also determined in both a neutral red uptake assay and a proliferation assay and appeared to be at least comparable to that of CDV, resulting in selective indices that were higher than those for CDV.

Previous reports suggested that (*N*)-MCT was a substrate of the HSV-1 TK, since an inhibitor of this enzyme reduced the phosphorylation of the drug (40), and the enzyme supplied in *trans* conferred sensitivity to the drug in uninfected cells (34). We hypothesized that the type II TK homologs expressed by orthopoxviruses might also be capable of activating this compound, since its structure was very close to the natural substrate of the enzyme. To test this hypothesis, we examined the antiviral activity of (*N*)-MCT against TK^+ and TK^- strains of CV and HSV-1 in a genetic resistance assay (27). Drugs that require TK for their activation exhibit elevated TK^-/TK^+ EC_{50} ratios, whereas compounds that do not require the enzymes to be active yield EC_{50} ratios near unity. In HSV-1, ACV was used as a positive control since it required phosphorylation by the TK and CDV was used as a negative control since it does not require phosphorylation by the enzyme to be active. The high EC_{50} ratios for ACV and idoxuridine (IDU) reflect the fact that they require phosphorylation by this enzyme (Table 2). (*N*)-MCT also yielded an elevated EC_{50} ratio, confirming that it was phosphorylated by TK, while the CDV negative control was unaffected by the deletion of this gene. In CV, IDU was used as a positive control since it was known to be activated by its TK, and CDV was used as a negative control. CDV was equally effective against the two viruses and yielded an EC_{50} ratio of 1.2, while IDU was much less effective against the TK^- strain of CV and yielded an EC_{50} ratio of 55 (Table 2). The TK^- virus was also significantly less sensitive to (*N*)-MCT and yielded an EC_{50} ratio of 14, suggesting that this compound may also require TK for its activation.

The ability of this compound to inhibit DNA synthesis was determined using CDV as a control. (*N*)-MCT was at least as effective in inhibiting VV DNA synthesis as the CDV positive control and yielded EC_{50}s of 1 and 3 μg/ml, respectively (Fig. 2). Both of these values were comparable to the antiviral EC_{50}s of 0.6 and 3 μg/ml, respectively, and were sufficient to explain the antiviral effects of the drug.

The antiviral activity of the compound in vitro taken together with its pharmacokinetic parameters (26) and the mechanism of action studies suggested that it might be an effective and highly selective compound in vivo. An established animal model was used to evaluate the antiviral activity of the compound against HSV-1 (20). BALB/c mice were infected i.n. with the E377 strain of HSV-1, and animals were treated twice daily beginning 24 h after infection. In this experiment, 7 of 14 animals succumbed to the infection, suggesting that the inoculum was slightly lower than the 90% lethal dose target dose. Nevertheless, all doses of the drug protected mice from mortality ($P < 0.01$) and were comparable to the ACV positive control (Table 3). These data suggested that this compound might be useful in the treatment of HSV infections.

Established models of orthopoxvirus infections were also used to evaluate the efficacy of this compound against lethal VV and CV infections in BALB/c mice (31). In the first study, groups of 15 animals were infected i.n. with a lethal dose of the

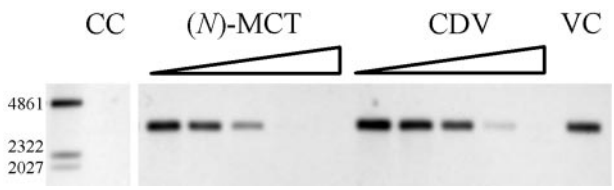

FIG. 2. Inhibition of VV DNA synthesis by (*N*)-MCT. Monolayers of HFF cells were infected with the WR strain of VV and incubated with increasing concentrations of the drugs as illustrated in the figure (0.03, 0.1, 3, 10, and 30 μg/ml). Purified DNA was cut with EcoRV, and a 3,259-bp fragment of VV DNA was detected with a digoxigenin-labeled DNA probe. Numbers at left are molecular sizes in base pairs. CC and VC designate cell control and virus control, respectively.

TABLE 3. Effect of treatment with (*N*)-MCT in BALB/c mice infected intranasally with HSV-1

Treatment[a]	Mortality			MDD[b]	*P* value
	No. dead/ total no.	%	*P* value		
Placebo					
0.4% CMC	7/14	50		8.0	
ACV					
50 mg/kg	0/15	0	<0.01		
16.7 mg/kg	0/15	0	<0.01		
5.6 mg/kg	0/15	0	<0.01		
(*N*)-MCT					
50 mg/kg	0/15	0	<0.01		NS[c]
16.7 mg/kg	0/15	0	<0.01		NS
5.6 mg/kg	1/15	7	<0.05	20	0.05

[a] (*N*)-MCT was prepared in 0.4% CMC and delivered i.p. twice daily in 0.1-ml doses. ACV was prepared in sterile water and given i.p. twice daily in 0.1-ml doses. All animals were treated for 7 days beginning 24 h postinfection.
[b] MDD, mean day of death.
[c] NS, not significant.

TABLE 4. Effect of treatment with (*N*)-MCT in BALB/c mice infected intranasally with VV or CV

Strain and treatment[a] (mg/kg)	Mortality			MDD[b]	*P* value
	No. dead/ total no.	%	*P* value		
VV strain IHD					
0.4% CMC	15/15	100		7.6	
CDV					
15	0/15	0	<0.001		
(*N*)-MCT					
50	0/15	0	<0.001		
16.7	0/15	0	<0.001		
5.6	3/15	20	<0.001	8.7	<0.05
VV strain WR					
0.4% CMC	15/15	100		7.9	
CDV					
15	0/15	0	<0.001		
(*N*)-MCT					
50	0/15	0	<0.001		
16.7	2/15	13	<0.001	7.5	NS[c]
5.6	12/15	80	NS	8.2	NS
CV strain Brighton					
0.4% CMC	15/15	100		9.6	
CDV					
15	0/15	0	<0.001		
(*N*)-MCT					
50	2/15	13	<0.001	7.5	NS
16.7	3/15	20	<0.001	13.3	0.01
5.6	6/15	40	<0.001	14.0	0.05

[a] (*N*)-MCT was prepared in 0.4% carboxymethyl cellulose and delivered i.p. twice daily in 0.1-ml doses. CDV was prepared in sterile saline and given i.p. once daily in 0.1-ml doses. All animals were treated for 5 days beginning 24 h postinfection.
[b] MDD, mean day of death.
[c] NS, not significant.

IHD strain of VV. Drug was administered i.p. twice daily at doses of 50, 16.7, and 5.6 mg/kg starting 24 h post-i.n. inoculation. Mortality in all mice treated with vehicle was 100%, whereas all mice treated with CDV at 15 mg/kg given once daily survived (Table 4). All animals treated with 50 or 16.7 mg/kg (*N*)-MCT administered twice daily survived, and 80% survived at the 5.6-mg/kg dose. All treatments resulted in significantly improved mortality compared to that of vehicle-treated controls ($P < 0.001$).

The successful treatment of the IHD strain of VV prompted us to repeat the experiment using the WR strain, which is a more pathogenic strain in our experimental model and has also been reported to be more lethal by others (38). All mice survived when treated with CDV at 15 mg/kg, and mortality was 100% in vehicle-treated control mice (Table 4). All animals treated with (*N*)-MCT at a dose of 50 mg/kg survived the infection, and mortality was 13% and 80% at the 16.7- and 5.6-mg/kg dose regimens given twice daily, respectively. In this experiment the drug significantly protected mice at the high and medium dose ($P < 0.001$) but there was no significant protection at the low dose. These data are consistent with the first experiment and also with the notion that the WR strain is a slightly more virulent virus in this model.

The same experiment was repeated against the Brighton strain of CV to confirm that (*N*)-MCT was active against another orthopoxvirus. In this experiment, the CDV control protected all animals from lethal infection with CV and all vehicle-treated animals died (Table 4). Animals treated at all doses of the test drug were significantly protected from mortality ($P < 0.001$), although a few animals died in each of the three treatment groups. These data taken together indicate that (*N*)-MCT is highly effective in treating orthopoxvirus infections in vivo.

DISCUSSION

Results presented here describe the antiviral activity of (*N*)-MCT against HSV-1, VV, and CV both in vitro and in vivo. This compound is unusual in that it exhibits TK dependence both in a herpesvirus and in an orthopoxvirus, and its rather uncommon spectrum of activity appears to reflect its selective phosphorylation in cells infected with these viruses. While many nucleoside analogs can be activated by the type I TK homologs encoded by some of the herpesviruses, few are apparently phosphorylated by the distinct type II TK molecules expressed by orthopoxviruses and including IDU, bromodeoxyuridine (10), and (*N*)-MCT as described here. A previous report suggested that the metabolism of this compound required the enzymatic activity of the HSV-1 TK and that the compound was converted to (*N*)-MCT triphosphate, which was incorporated in cellular DNA by the host polymerase (22). These data taken together with results presented here suggest that the compound may inhibit both orthopoxvirus and herpesvirus DNA polymerases and impair viral DNA synthesis by a similar mechanism. Alternative interpretations of the data exist, and we cannot exclude the possibility that (*N*)-MCT interferes with orthopoxvirus DNA synthesis indirectly through the inhibition of thymidylate synthetase by the monophosphate form of the drug. It is also possible, albeit unlikely, that differences in drug activities against CV are caused by genetic differences outside the TK locus. Although additional experiments will be required to confirm directly the inhibition of the VV DNA polymerase by (*N*)-MCT triphosphate, the active site is highly conserved between this enzyme and its homolog in herpes simplex virus.

This proposed mechanism of action is interesting in that it

appears to be sufficient to explain the spectrum of activity of this compound. It is active against the orthopoxviruses and the alphaherpesviruses since they express enzymes with TK activity. The gammaherpesvirus Epstein-Barr virus also encodes a TK homolog, and the drug inhibits its replication with an EC_{50} of 0.45 μg/ml (data not shown). Human herpesvirus 8 also expresses a TK homolog and also appears to be sensitive to the drug (41). In contrast, this compound appears to be inactive against the betaherpesviruses (human cytomegalovirus and human herpesvirus 6) which do not express an enzyme with TK activity, an observation which is also consistent with this mode of action. The property of TK dependence is significant since it should confer the desirable characteristics of good antiviral activity and minimal toxicity in vivo. Data from animal models of lethal orthopoxvirus and HSV-1 infections presented here appear to bear out this prediction. We conclude that this compound holds promise, and additional experiments are warranted to continue its development as a potential therapy for orthopoxvirus and herpesvirus infections.

In summary, (*N*)-MCT is a compound with an interesting spectrum of activity that is conferred by its selective phosphorylation by some herpesvirus and orthopoxvirus TK homologs. This interesting property might be useful in that the drug could be used to treat both herpesvirus and orthopoxvirus infections. Additional experiments will be required to improve its oral bioavailability and to characterize rigorously its toxicity profile. The completion of these studies will help establish the clinical potential of (*N*)-MCT and will be important milestones in the development of this compound as a potential therapy for the treatment of herpesvirus and orthopoxvirus infections.

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