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# Efficacy of *N*-methanocarbathymidine in treating mice infected intranasally with the IHD and WR strains of vaccinia virus

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**Abstract**

*N*-Methanocarbathymidine [(*N*)-MCT] is a newly identified inhibitor of orthopoxvirus replication in cell culture and in mice. Limited published animal studies indicated the compound is effective by intraperitoneal (i.p.) route at 10–100 mg/(kg day). More extensive studies using different treatment regimens in intranasally infected mice were conducted in order to further explore the potential of this compound compared to cidofovir in treating vaccinia virus infections. (*N*)-MCT was given twice a day for 7 days, whereas cidofovir was administered once a day for 2 days, each starting 24 h after virus exposure for most experiments. (*N*)-MCT was not toxic up to 1000 mg/(kg day) by the i.p. treatment route. Oral and i.p. treatment regimens with (*N*)-MCT were directly compared during a vaccinia virus (IHD strain) infection, indicating that the nucleoside has good oral bioavailability in mice. Treatments by i.p. route with (*N*)-MCT (100 mg/(kg day)) reduced lung, nasal, and brain virus titers during an IHD virus infection, but not nearly to the same extent as i.p. cidofovir (100 mg/(kg day)). Treatment with both compounds decreased liver, spleen, and kidney virus titers, as well as reduced lung consolidation scores and lung weights. Onset of treatment could be delayed by 2 days with (*N*)-MCT and by 3 days with cidofovir, providing significant survival benefit during the IHD virus infection. Against a vaccinia virus (WR strain) infection in mice, i.p. (*N*)-MCT treatment prevented death at 500 mg/(kg day), which was comparable in activity to i.p. cidofovir (100 mg/(kg day)). Significant reductions in tissue virus titers occurred with both treatment regimens. (*N*)-MCT could be further pursued for its potential to treat orthopoxvirus infections in humans.


*Keywords:* Antiviral; Orthopoxvirus; Vaccinia virus; (*N*)-MCT; Cidofovir; Thymidine

## 1. Introduction

Antiviral treatments are being sought against infections caused by orthopoxviruses and parapoxviruses, including complications due to smallpox vaccination (Bray, 2003), molluscum contagiosum (Baxter and Highet, 2004; Arican, 2006; Brown et al., 2006), orf viruses (De Clercq, 2002; McCabe et al., 2003; Erbağci et al., 2005), or resulting from acts of bioterrorism (Baker et al., 2003; Jahrling et al., 2005). In certain infections antiviral treatment has been shown to be superior to vaccination (Stittelaar et al., 2006). Cidofovir, a nucleotide analog, and cidofovir prodrugs are leading candidates for treatment of poxvirus infections, and have been investigated in various animal models (Bray et al., 2000; De Clercq, 2002; Smee and Sidwell, 2003; De Clercq and Neyts, 2004; Quenelle et al., 2003, 2004; Smee et al., 2004). Cidofovir acts by inhibiting viral DNA polymerase activity (Magee et al., 2005). Due to toxicity and the lack of oral bioavailability of cidofovir (Wachsman et al., 1996), it would be desirable to identify other inhibitory substances that might be useful in treating poxvirus infections. Recently a low molecular weight compound, 4-trifluoromethyl-*N*-(3,3a,4,4a,5,5a,6,6a-octahydro-1,3-dioxo-4,6-ethenocycloprop[*f*]isoindol-2(1*H*)-yl)-benzamide (ST-246), was reported to possess potent anti-poxvirus activity in cell culture and in mice (Yang et al., 2005). ST-246 is not a nucleoside/nucleotide analog, and inhibits virus replication by inhibiting a step involved in virus particle maturation to the extracellular form. Thus, it represents a potential drug candidate with a mode of action different from that of cidofovir.

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We have evaluated a number of potential anti-poxvirus agents exhibiting antiviral activity in vitro, most of which are 2′-deoxynucleoside/nucleotide analogs and generally target the viral DNA polymerase. One such compound, *N*-methanocarbathymidine [(*N*)-MCT, also referred to as (north)-methanocarbathymidine (Marquez et al., 1996; Zalah et al., 2002), *N*-methanocarbathymine or (north)-methanocarbathymine (Russ et al., 2003)], was shown to inhibit cowpox and vaccinia viruses in cell culture and infected mice (Prichard et al., 2006; Smee et al., 2007). It also inhibits herpes viruses (Marquez et al., 1996; Zalah et al., 2002; Russ et al., 2003; Huleihel et al., 2005) by first undergoing phosphorylation in infected cells (Zalah et al., 2002; Marquez et al., 2004; Schelling et al., 2004) and inhibiting the viral DNA polymerase at the nucleoside triphosphate level (Marquez et al., 2004). (*N*)-MCT is a carbocyclic analog of thymidine. Its spectrum of anti-poxvirus activity is similar to that of carbocyclic thymidine (Smee et al., 2007).

We found (*N*)-MCT to be non-toxic to mice at high doses, and thus is an interesting candidate for the treatment of poxvirus infections. Previously (*N*)-MCT was investigated in mice at doses of 10–100 mg/(kg day), principally by intraperitoneal treatment route (Prichard et al., 2006; Smee et al., 2007). At these doses, vaccinia (WR strain) virus was less affected by antiviral treatment than were vaccinia (IHD strain) and cowpox viruses. The objectives of the present research were to determine the oral efficacy of (*N*)-MCT, and to study different treatment regimens against vaccinia virus infections in mice. Particularly, we were interested in defining regimens that may be more effective in preventing death, reducing virus burden, and establishing how late after infection treatments could be initiated and still be effective.

## 2. Materials and methods

### 2.1. Viruses and test compounds

Vaccinia viruses (IHD and WR strains) were purchased from the American Type Culture Collection, Manassas, VA. The viruses were propagated in African green monkey kidney (MA-104) cells (BioWhittaker, Walkersville, MD) for use in these studies. (*N*)-MCT was provided to us through the NIAID antiviral testing program. Cidofovir was obtained from Mick Hitchcock (Gilead Sciences, Foster City, CA). Sterile water was used to dissolve (*N*)-MCT up to 100 mg/(kg day) for oral dosing. Studies with higher doses required formulating the compound in water containing 20% (2-hydroxypropyl)-β-cyclodextrin (HPCD, Sigma, St. Louis, MO). Sterile saline containing 20% HPCD was used for high-dose intraperitoneal (i.p.) treatments. The placebo controls received the same HPCD formulations. Cidofovir in sterile saline was given by i.p. route.

### 2.2. Animal experiment design

Female BALB/c mice (13–15 g, from Charles River Laboratories, Wilmington, MA) were anesthetized with Ketamine (100 mg/kg) by i.p. injection. They were infected intranasally with approximately $1 \times 10^5$ plaque forming units of vaccinia virus per mouse in a 50-μl inoculum volume. The animals were treated orally by gavage or i.p. with (*N*)-MCT, or i.p. with cidofovir in a 100 μl volume. Cidofovir is not effective orally due to very low oral bioavailability (Wachsman et al., 1996). Treatments with (*N*)-MCT were given twice a day (12 h apart) for 7 days whereas cidofovir (100 mg/(kg day)) was administered once a day for 2 days. These regimens have previously been shown to be effective for treating poxvirus-infected mice (Smee et al., 2007). Treatments usually started 24 h after virus challenge, or as indicated in one of the tables. The placebo was given in parallel with the (*N*)-MCT group. Animals were observed 21 days for death.

Virus titer determinations were made from tissues taken from groups of five infected mice held for this purpose during the infection. Homogenization of soft tissues was done in 1 ml of cell culture medium (MEM, 0.18% sodium bicarbonate, 50 μg/ml gentamicin) using a stomacher. Snouts and accompanying nasal tissues were ground in 1 ml of medium using sterilized mortars and pestles. Samples were serially diluted in 10-fold increments and plaque titrated in rhesus monkey kidney LLC-$MK_2$ cells (obtained from the American Type Culture Collection, Manassas, VA). Plaques were stained at 3 days with 0.2% crystal violet in 10% buffered formalin, then were counted with the aid of a light box. Plaque numbers were converted to plaque forming units (PFU) per gram of lung tissue. Because the severe infections also induced pneumonitis, we were able to report lung consolidation scores (graded from 0 to 4) and lung weights as part of the experiment.

### 2.3. Statistical evaluation

The two-tailed Fisher exact test was used to evaluate survivor number increases. The two-tailed Mann–Whitney *U*-test was used to analyze differences in the mean day of death, lung infection parameters (consolidation score and weight), and tissue virus titers. All statistical comparisons were made between drug-treated groups and the placebo control.

## 3. Results

### 3.1. Toxicity of (N)-MCT in uninfected mice

(*N*)-MCT was administered i.p. twice a day for 7 days at doses of 100, 250, 500, 750 and 1000 mg/(kg day). Animal weights were recorded daily for 21 days. Mice treated with all doses of (*N*)-MCT gained weight in parallel with placebo-treated mice, indicating a lack of toxicity of the compound up to 1000 mg/(kg day). Doses as high as 500 mg/(kg day) were selected for antiviral experiments.

### 3.2. Oral activity of (N)-MCT against vaccinia virus (IHD strain) infections in mice

Previously (*N*)-MCT was found to be highly effective when given by i.p. route against vaccinia virus (IHD strain) infections (Prichard et al., 2006; Smee et al., 2007), with complete protection at 10, 30, and 100 mg/(kg day) reported in the latter study.

Table 1
Effects of i.p. and oral treatment with (*N*)-MCT on a vaccinia virus (IHD strain) respiratory infection in mice

| Compound (mg/(kg day))[a] | Treatment route | Survivors/total | MDD[b] |
|---|---|---|---|
| Experiment 1 | | | |
| (*N*)-MCT (100) | i.p. | 10/10*** | – |
| (*N*)-MCT (30) | i.p. | 9/10*** | 7.0 |
| (*N*)-MCT (10) | i.p. | 3/10* | 10.7 ± 3.9*** |
| Placebo | i.p. | 0/20 | 6.8 ± 0.5 |
| (*N*)-MCT (100) | p.o. | 10/10*** | – |
| (*N*)-MCT (30) | p.o. | 9/10*** | 8.0 |
| (*N*)-MCT (10) | p.o. | 7/10*** | 8.7 ± 1.2* |
| Placebo | p.o. | 0/20 | 7.1 ± 0.7 |
| Experiment 2 | | | |
| (*N*)-MCT (100) | i.p. | 10/10*** | – |
| (*N*)-MCT (30) | i.p. | 3/10 | 8.6 ± 1.6* |
| (*N*)-MCT (10) | i.p. | 1/10 | 8.6 ± 1.2* |
| (*N*)-MCT (3) | i.p. | 3/10 | 8.9 ± 0.7** |
| Placebo | i.p. | 0/10 | 7.0 ± 0.9 |
| (*N*)-MCT (100) | p.o. | 7/10** | 8.3 ± 0.6* |
| (*N*)-MCT (30) | p.o. | 8/10*** | 7.5 ± 0.7 |
| (*N*)-MCT (10) | p.o. | 0/10 | 7.8 ± 0.4 |
| (*N*)-MCT (3) | p.o. | 0/10 | 7.4 ± 0.5 |
| Placebo | p.o. | 0/10 | 7.1 ± 0.6 |

*$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$.
[a] Twice-daily (12 h apart) for 7 days starting 24 h after virus exposure.
[b] Mean day of death ± S.D. of mice that died prior to day 21.

To extend these observations, treatments were performed against the IHD infection by directly comparing i.p. and oral routes of administration evaluated at the same time (Table 1). Oral and i.p. doses at 100 and 30 mg/(kg day) gave nearly the same degree of protection (Experiment 1). At 10 mg/(kg day), the oral and i.p. treatments gave 70% and 30% protection, respectively. These results at 10 mg/(kg day) were not statistically significantly different from each other, however. There was a considerable delay in death in the 10 mg/(kg day) i.p. group. To confirm these findings, a repeat experiment was conducted, and the results are presented in Table 1, Experiment 2. In this study (*N*)-MCT was not as protective as in the first test. Both oral and i.p. doses were protective at the 100 mg/(kg day) dose, but oral treatment was more active than i.p. administration at the 30 mg/(kg day) dose. Neither treatment was significantly effective at the 10 and 3 mg/(kg day) doses. In both experiments the results indicate that (*N*)-MCT is effective by the oral route in mice.

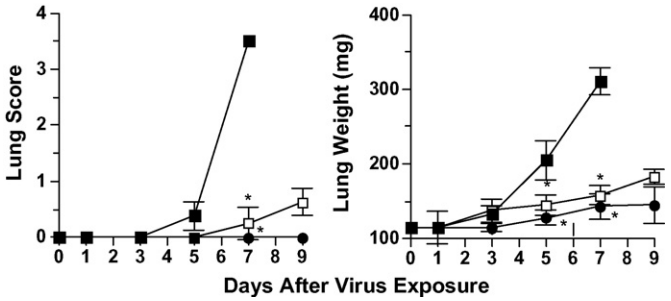


Fig. 1. Effects of i.p. treatment with (*N*)-MCT and cidofovir on mean lung consolidation scores and lung weights during a vaccinia virus (IHD strain) respiratory infection in mice. Treatment with (*N*)-MCT was given twice a day (12 h apart) for 7 days. Treatment with cidofovir was administered once a day for 2 days. Treatment began at 24 h after virus exposure. There were five mice per group. Symbols—(open squares) (*N*)-MCT (100 mg/(kg day)); (circles) cidofovir (100 mg/(kg day)); (filled squares) placebo. *$P < 0.05$.

A follow-up experiment was conducted with (*N*)-MCT at higher doses compared to cidofovir at 100 mg/(kg day) to assess effects on mortality and lung infection parameters (Table 2). Survival benefit was afforded by oral (*N*)-MCT treatments of 100, 250, and 500 mg/(kg day), and by i.p. cidofovir at 100 mg/(kg day). The two higher doses of (*N*)-MCT were superior to the 100 mg/(kg day) dose in reducing lung virus titers, but virus titer reduction was not as great as that resulting from cidofovir treatment. Both compounds were effective in significantly reducing lung consolidation scores and lung weights on day 5 of the infection.

### 3.3. Effects of treatment on pathogenesis of a vaccinia virus (IHD strain) infection

(*N*)-MCT did not have a large impact in reducing lung virus titers by either oral treatment or by the i.p. route (Smee et al., 2007) when compared to cidofovir, as determined on day 5 of the infections. The pathogenesis of the vaccinia virus (IHD strain) infection was studied over several days using an i.p. dose of 100 mg/(kg day). Lung consolidation score and lung weight parameters of the infection are shown in Fig. 1. Lung scores and weights were elevated in the placebo group on days 5 and 7 of the infection, being highest just prior to death of the animals. (*N*)-MCT and cidofovir treatments resulted in sig-

Table 2
Effects of oral treatment with (*N*)-MCT and i.p. treatment with cidofovir on vaccinia virus (IHD strain) respiratory infections in mice

| Compound (mg/(kg day))[a] | Treatment days | Survivors/total | MDD[b] | Mean lung parameters ± S.D. (day 5) | | |
|---|---|---|---|---|---|---|
| | | | | Score | Weight (mg) | Virus titer[c] |
| (*N*)-MCT (500) | 1–7 | 10/10*** | – | 0.0 ± 0.0** | 134 ± 11** | 6.7 ± 0.3** |
| (*N*)-MCT (250) | 1–7 | 10/10*** | – | 0.0 ± 0.0** | 122 ± 9** | 6.8 ± 0.4** |
| (*N*)-MCT (100) | 1–7 | 9/10*** | 8.0 | 0.3 ± 0.3** | 160 ± 29** | 7.5 ± 0.2** |
| Cidofovir (100) | 1–2 | 10/10*** | – | 0.0 ± 0.0** | 114 ± 11** | 4.5 ± 0.5** |
| Placebo | 1–7 | 0/20 | 7.0 ± 1.9 | 1.4 ± 0.2 | 252 ± 20 | 8.1 ± 0.3 |

**$P < 0.01$ and ***$P < 0.001$.
[a] Twice-daily (12 h apart) for (*N*)-MCT and once-daily for cidofovir, starting 24 h after virus exposure.
[b] Mean day of death ± S.D. of mice that died prior to day 21.
[c] $\log_{10}$ PFU/g ± S.D.

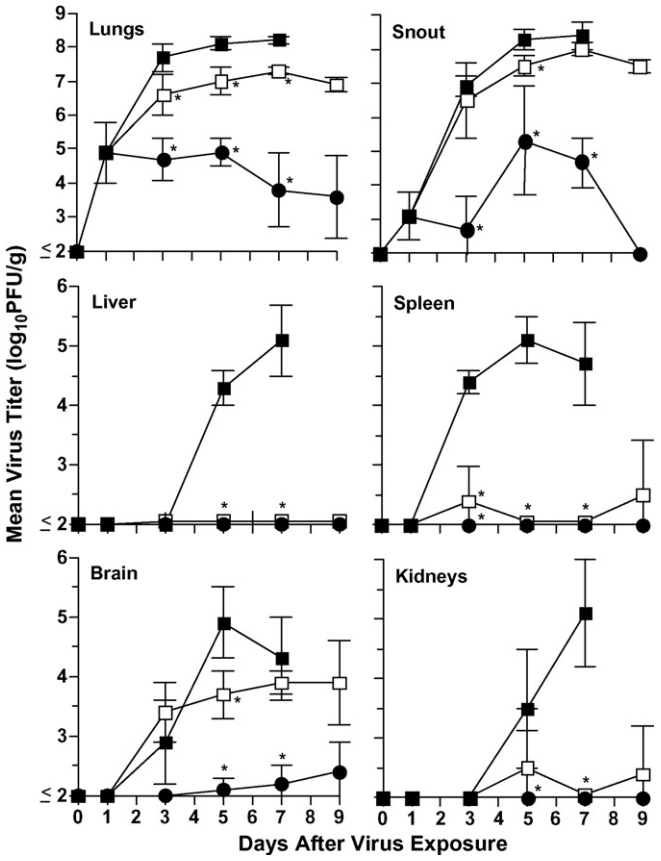


Fig. 2. Effects of i.p. treatment with (*N*)-MCT and cidofovir on mean tissue virus titers during a vaccinia virus (IHD strain) respiratory infection in mice. Treatment with (*N*)-MCT was given twice a day (12 h apart) for 7 days. Treatment with cidofovir was administered once a day for 2 days. Treatment began 24 h after virus exposure. There were five mice per group. Symbols—(open squares) (*N*)-MCT (100 mg/(kg day)); (circles) cidofovir (100 mg/(kg day)); (filled squares) placebo. $^{*}P < 0.05$.

nificant decreases in these parameters relative to the placebo group. The effects of cidofovir appeared to be slightly better than those of (*N*)-MCT in reducing these lung infection parameters, although the differences in the results were not statistically significant.

The effects of antiviral treatment on virus titers in six different tissues during the IHD virus infection are presented in Fig. 2. On days 3, 5, and 7, (*N*)-MCT and cidofovir treatments at a dose of 100 mg/(kg day) caused significant reductions in lung virus titers. Lung virus titers were reduced by about 10-fold in the (*N*)-MCT-treated mice and by ≥1000-fold in cidofovir-treated animals. Virus in nasal tissue was significantly reduced (about sixfold) by (*N*)-MCT treatment on day 5 of the infection. Cidofovir treatment caused ≥1000-fold nasal virus titer reductions on days 3, 5, and 7. Virus in liver, spleen, and kidneys became detectable in placebos starting on day 3. Both (*N*)-MCT and cidofovir dramatically reduced virus titers in these tissues, and to nearly the same extent. (*N*)-MCT reduced brain virus titers significantly (by 16-fold) on day 5 of the infection, whereas cidofovir was effective on days 5 and 7, causing virus titer reductions of 630- and 125-fold, respectively. The results demonstrate that (*N*)-MCT reduced lung, snout, and brain virus titers over multiple days of the infection, but to a lesser extent than cidofovir.

Table 3
Effects of delayed i.p. treatment with (*N*)-MCT and cidofovir on a vaccinia virus (IHD strain) respiratory infection in mice

| Compound[a] (mg/(kg day)) | Treatment start day[b] | Survivors/ total | MDD[c] |
|---|---|---|---|
| (*N*)-MCT (100) | 1 | 8/10*** | 11.0 ± 1.4* |
| Cidofovir (100) | 1 | 10/10*** | – |
| Placebo | 1 | 0/10 | 6.7 ± 0.5 |
| (*N*)-MCT (100) | 2 | 5/10* | 11.2 ± 1.8*** |
| Cidofovir (100) | 2 | 10/10*** | – |
| Placebo | 2 | 0/10 | 6.5 ± 0.5 |
| (*N*)-MCT (100) | 3 | 2/10 | 15.5 ± 6.3* |
| Cidofovir (100) | 3 | 7/10** | 15.3 ± 4.7** |
| Placebo | 3 | 0/10 | 7.4 ± 0.7 |
| (*N*)-MCT (100) | 4 | 1/10 | 7.2 ± 0.7 |
| (*N*)-MCT (400) | 4 | 0/10 | 7.0 ± 0.7 |
| Cidofovir (100) | 4 | 3/10 | 8.9 ± 2.3* |
| Placebo | 4 | 0/10 | 6.9 ± 0.7 |

$^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.
[a] Twice-daily (12 h apart) for 7 days with (*N*)-MCT and once-daily for 2 days with cidofovir.
[b] After virus exposure.
[c] Mean day of death ± S.D. of mice that died prior to day 21.

### 3.4. Effects of delayed treatment on survival from vaccinia virus (IHD strain) infection

Treatments with (*N*)-MCT and cidofovir by i.p. route were initiated at varying times after infection to determine their impact on survival (Table 3). (*N*)-MCT at 100 mg/(kg day) was significantly effective in preventing mortality when treatment started at 1 and 2 days after virus exposure. Delays in the mean day of death occurred when treatment started as late as 3 days after infection. Treatment with (*N*)-MCT was also performed using 400 mg/(kg day) starting on day 4 of the infection. This treatment had no impact on survival. Cidofovir (100 mg/(kg day)) significantly protected mice from death when treatment started as late as 3 days after infection, and delayed the time to death when treatment started at 4 days post-virus exposure.

### 3.5. Treatment of a vaccinia virus (WR) strain infection in mice

It was previously demonstrated that the vaccinia (WR strain) infection was more difficult to treat with (*N*)-MCT than was the IHD strain infection in terms of survival benefit (Prichard et al., 2006; Smee et al., 2007). The i.p. doses tested were 10–100 mg/(kg day). We evaluated (*N*)-MCT at higher i.p. doses in an attempt to provide a better response to treatment (Table 4). (*N*)-MCT reduced mortality significantly at 500 mg/(kg day) but not at the two lower doses. Mice dying from the infection lived significantly longer than placebos when treated with 250 and 500 mg/(kg day) doses of (*N*)-MCT. Cidofovir at 100 mg/(kg day) protected all mice from death. Lung consolidation scores and lung weights were significantly reduced by treatment with both compounds.

Table 4
Effects of i.p. treatment with (*N*)-MCT and cidofovir on survival and lung infection parameters during a vaccinia virus (WR strain) respiratory infection in mice

| Compound (mg/(kg day))[a] | Treatment (days) | Survivors/total | MDD[b] | Mean lung parameters ± S.D. (day 5) | |
|---|---|---|---|---|---|
| | | | | Score | Weight (mg) |
| (*N*)-MCT (500) | 1–7 | 8/10*** | 11.5 ± 2.1** | 0.0 ± 0.0** | 140 ± 25** |
| (*N*)-MCT (250) | 1–7 | 2/10 | 9.4 ± 2.2*** | 0.1 ± 0.2* | 162 ± 16** |
| (*N*)-MCT (100) | 1–7 | 2/10 | 6.3 ± 2.4 | 0.2 ± 0.3* | 156 ± 38* |
| Cidofovir (100) | 1–2 | 10/10*** | – | 0.2 ± 0.3* | 134 ± 11** |
| Placebo | 1–7 | 0/20 | 7.0 ± 0.5 | 0.7 ± 0.3 | 216 ± 21 |

*$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$.
[a] Twice-daily (12 h apart) for (*N*)-MCT and once-daily for cidofovir, starting 24 h after virus exposure.
[b] Mean day of death ± S.D. of mice that died prior to day 21.

Tissue virus titers were determined on day 5 of the vaccinia virus (WR strain) infection (Fig. 3). The 500 mg/(kg day) dose of (*N*)-MCT was equal to that of cidofovir in reducing lung virus titers. The other two doses on (*N*)-MCT also significantly reduced lung virus titers. Differences in activity between cidofovir and (*N*)-MCT were not statistically significant. Treatment with the compounds markedly reduced liver virus production, and virus titers were also reduced in the spleen. The 500 mg/(kg day) dose of (*N*)-MCT and cidofovir (100 mg/(kg day)) reduced brain virus titers significantly.

## 4. Discussion

The results indicate that (*N*)-MCT is effective in treating infections caused by the IHD and WR strains of vaccinia virus in mice using various treatment regimens. The potency of (*N*)-MCT is less than that of cidofovir, since (*N*)-MCT required daily treatment for a longer period of time than cidofovir (7 days versus 2 days of treatment in this study). However, (*N*)-MCT appears to be less toxic than cidofovir, at least in mice, based upon our toxicity data, and was well tolerated at the highest dose tested of 1000 mg/(kg day).

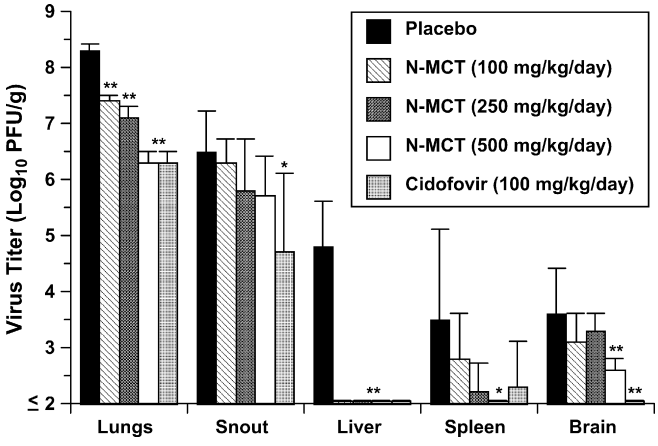


Fig. 3. Effects of i.p. treatment with (*N*)-MCT and cidofovir on mean tissue virus titers, determined on day 5 of a vaccinia virus (WR strain) respiratory infection in mice. Treatment with (*N*)-MCT was given twice a day (12 h apart) for 7 days. Treatment with cidofovir was administered once a day for 2 days. Treatment began 24 h after virus exposure. There were five mice per group. *$P < 0.05$, **$P < 0.01$.

The oral activity of (*N*)-MCT was judged to be similar to that of the i.p. dosing regimen based upon survival benefit. Variability in survival benefit from experiment to experiment was seen at the lower doses. The reason for this is unclear, but may relate to the fact that (*N*)-MCT is only weakly inhibitory to lung virus titers at the 10 and 30 mg/(kg day) doses (Smee et al., 2007). Thus, the factors determining survival and death at these doses in different experiments may be small. For this reason it was important to evaluate oral versus i.p. treatments in the same experiment. Pharmacokinetic studies will need to be conducted to accurately determine oral bioavailability, which to our knowledge have not yet been conducted in any laboratory.

(*N*)-MCT was less active than cidofovir in its ability to suppress virus titers in the lungs, snout, and brain of mice infected with vaccinia virus. The highest dose of (*N*)-MCT (500 mg/(kg day)) showed an improvement in efficacy over the lower doses. Both (*N*)-MCT and cidofovir were able to substantially block the spread of virus to the liver and spleen. There was a correlation between high nasal virus titer and elevated titers in the brain. Because cidofovir reduced nasal virus titer more effectively than (*N*)-MCT, this may have been the reason for the presence of less virus in the brains of cidofovir-treated mice.

Treatment with (*N*)-MCT could be delayed by 48 h and still show a beneficial impact on survival. Cidofovir was still beneficial when treatment started as late as 72 h after virus exposure. A high dose of (*N*)-MCT starting at 4 days was not beneficial, indicating that the window of effective treatment had passed, and the mice could not be rescued by a high dose of compound.

(*N*)-MCT was effective at 500 mg/(kg day) in preventing death in mice infected with the WR strain of vaccinia virus. The compound was reported active at 100 mg/(kg day) previously (Prichard et al., 2006; Smee et al., 2007). The WR virus is highly virulent to mice, and antiviral results from different experiments may vary. The effects of (*N*)-MCT at 500 mg/(kg day) were somewhat comparable to those of cidofovir at 100 mg/(kg day). Infections with the IHD strain of virus have been more consistently treatable with antiviral compounds (Smee et al., 2004, 2007).

It was previously demonstrated in cell culture studies that the potency of (*N*)-MCT was highly dependent on the cell line (Smee et al., 2007). The compound was particularly potent in mouse cells, but much less effective in cells of higher species of animals. Its activity was much lower in human and monkey

cells, for example. Work in higher species such as monkeys will be needed to determine the potential utility of treating poxvirus infections in humans with (*N*)-MCT.

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