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Short Communication

N-Methanocarbathymidine is more effective than acyclovir for treating neonatal herpes simplex virus infection in guinea pigs

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ABSTRACT

The outcome of neonatal herpes simplex (HSV) infection, even after therapy with high dose acyclovir (ACV), is not optimum. We therefore evaluated *N*-Methanocarbathymidine ((*N*)-MCT) using the guinea pig model of neonatal herpes. Treatment with ACV (60 mg/kg/day) was compared to doses of 1, 5, and 25 mg/kg/day of (*N*)-MCT initiated 1, 2, or 3 days postinoculation (dpi). Both ACV and (*N*)-MCT significantly improved survival, but only (*N*)-MCT significantly reduced the number of animals with symptoms when begun at 1 dpi. When therapy was begun at 2 dpi, only (*N*)-MCT (1, 5, or 25 mg/kg/day) significantly increased survival. In fact, (*N*)-MCT improved survival up to 3 dpi, the last time point evaluated. (*N*)-MCT was highly effective and superior to high dose ACV therapy for the treatment of neonatal herpes in the guinea pig model.

Neonatal herpes simplex virus (HSV) infections are rare, about 1500 cases/year, but have devastating consequences for the newborn (Corey and Wald, 2009; Kimberlin, 2007). Following infection, the disease may be limited to the skin, eye, and/or mouth (SEM disease). However, both treated and especially untreated infections may progress to involve multiple organs including the liver, lung, adrenal glands (disseminated disease) and the brain (CNS disease) (Whitley et al., 1988). Current therapy with high dose (60 mg/kg/day) acyclovir (ACV) is more effective than vidarabine (Whitley et al., 1980) and lower dose (30 mg/kg/day) ACV (Skoldenberg et al., 1984; Whitley et al., 1991a). However, the reduction in morbidity and mortality for CNS and disseminated disease is still not optimal (Kimberlin et al., 2001a). High dose ACV is also commonly associated with neutropenia that can require treatment to be halted (Kimberlin et al., 2001a). Therefore, it is clear that there is a need for improved therapy.

N-Methanocarbathymidine ((*N*)-MCT) is a thymidine analog that incorporates a pseudosugar with a fixed Northern conformation. It has previously been shown to have potent antiviral activity against HSV-1 and HSV-2 (Marquez et al., 2006; Prichard et al., 2006; Quenelle et al., 2010; Zalah et al., 2002). The compound is selectively phosphorylated by the HSV thymidine kinase (TK) (Schelling et al., 2004) but phosphorylation is different from ACV. The HSV-1 TK catalyzes the conversion of (*N*)-MCT monophosphate to (*N*)-MCT diphosphate, whereas for ACV it creates the monophosphate (Zalah et al., 2002). (*N*)-MCT has a 1000-fold-greater affinity for the HSVTK than the human homolog and thus is highly selective for virus-infected cells (Prichard et al., 2006; Prota et al., 2000). We chose to evaluate (*N*)-MCT in the guinea pig model of neonatal herpes because it closely mimics human disease (Bravo et al., 1994, 1996). After infection, animals develop SEM disease with or without CNS and systemic involvement with a high but not universal mortality.

For these experiments, (*N*)-MCT was kindly provided by N&N Scientific Co. (Rockville, MD) and dissolved in saline (Prichard et al., 2006). ACV was obtained from Sigma Co. (St. Louis, MO). Female Hartley guinea pigs (250–350 g) were obtained from Charles River Breeding Laboratories (Wilmington, MA) and housed under AAALAC approved conditions. We used HSV-2 strain MS (ATCC-VR540) as previously described (Bravo et al., 1994). Newborn guinea pigs were inoculated intranasally within 48 h after birth with 8.7×10^5 pfu of HSV-2 (Bravo et al., 1994, 1996). Animals were randomly assigned within litters to receive doses of 1, 5, or 25 mg/kg/day of (*N*)-MCT, placebo (saline) or the recommended dose of ACV for neonates, 60 mg/kg/day (Bravo et al., 1994, 1996). Treatments were administered intraperitoneally (IP), twice daily for 10 days beginning 1–3 days post inoculation (dpi).

Animals were followed daily for clinical evidence of herpetic disease which consist of vesicular lesions on the nose, mouth or eyelids (skin, eye and mouth, or SEM disease). Animals were also evaluated for the presence of respiratory symptoms (nasal flaring,

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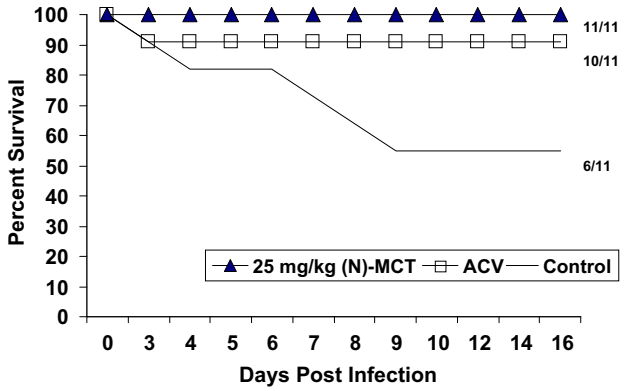

Fig. 1. Effect of *N*-methanocarbathymidine ((*N*)-MCT) treatment on neonatal HSV-2 infection in the guinea pig. Animals were treated with 25 mg/kg/day of (*N*)-MCT or 60 mg/kg/day of ACV beginning one day after virus inoculation and followed for 14 days. The numbers on the right show the number of surviving animals for each group.

subcostal or intercostal retractions, decreased respiratory rate) and general signs of disease (weight loss, ruffled fur, lethargy and dehydration (Bravo et al., 1994, 1996).

Based on the results obtained in previous mouse evaluations (data not shown), we selected a dose of 25 mg/kg/day of (*N*)-MCT begun at 1 dpi for the initial evaluation. As seen in Fig. 1, (*N*)-MCT treatment significantly increased survival of newborn guinea pigs compared to the control group (11/11 vs. 6/11, $p = 0.035$) and was equivalent to ACV (10/11). However, only 2/11 newborns treated with (*N*)-MCT exhibited any sign of infection compared to 9/11 newborns with symptoms in the ACV group ($p = 0.009$) (Table 1). We next evaluated a delay in the onset of drug therapy to 2 dpi. As seen in Table 1 (experiment 2), 25 mg/kg/day (*N*)-MCT remained protective, while ACV was not protective, similar to previous results in this model (Bravo et al., 1994, 1996). This experiment was followed by evaluations of lower doses of (*N*)-MCT and extended delays in initiation of therapy. For simplicity and because the mortality in the placebo group was consistent, we combined the results for the last two experiments which evaluated doses of 25 mg/kg of (*N*)-MCT initiated at 3 dpi, 5 mg/kg/day of (*N*)-MCT initiated at 2 and 3 dpi, and 1 mg/kg/day of(*N*)-MCT initiated at 2 dpi. A control group with ACV was not included since ACV therapy begun at 2 dpi was not protective in this model (above and Bravo et al., 1994, 1996). As seen in Table 2, the 25 mg/kg/day of (*N*)-MCT dose remained effective when therapy was begun at 3 dpi, with 100% survival (6/6, $p = 0.009$ vs. control). Similarly, the 5 mg/kg/day dose of (*N*)-MCT initiated at 2 dpi was also 100% protective (11/11 survival, $p = 0.001$ vs. control), and also protected most newborns from death (8/10, $p < 0.05$ vs. control) when treatment was begun at 3 dpi. Lastly, even a dose of 1 mg/kg/day of (*N*)-MCT provided significant protection (8/9 survival, $p = 0.009$ vs. control) when begun at 2 dpi. Significant decreases in the number of newborns exhibiting symptoms was, however, only seen in the group receiving 5 mg/kg/day of (*N*)-MCT when treatment was begun at 2 dpi (Table 2). For example, SEM disease and respiratory symptoms were seen in all placebo recipients but in only 4/11 (36.4%, $p = 0.004$ vs. placebo) and 2/11 (18.2%, $p = 0.0002$ vs. placebo) (respectively for SEM disease and respiratory symptoms) of the animals treated with 5 mg/kg/day of (*N*)-MCT beginning at 2 dpi.

Although high dose ACV therapy significantly improves the outcome of neonatal HSV infections, the morbidity and mortality remain unacceptably high (Corey and Wald, 2009; Kimberlin, 2007; Kimberlin et al., 2001a,b). In the investigations presented here, we investigated a new drug with potent anti-HSV activity, (*N*)-MCT using our guinea pig model of neonatal herpes (Bravo et al., 1994, 1996). The guinea pig model of neonatal HSV mimics human disease with the development of local vesicular disease (SEM disease) which progresses to systemic and CNS infection with high but not universal mortality if untreated (Bravo et al., 1994). Because the outcome of neonatal HSV infection has been correlated to the time of initiation of therapy in relation to symptoms onset (Whitley et al., 1991b), we compared (*N*)-MCT to ACV at various times post HSV infection.

In these experiments, we found that (*N*)-MCT at doses of 5 or 25 mg/kg/day significantly decreased mortality when begun as late as 3 dpi while ACV at 60 mg/kg/day was only effective when initiated within 1 day of virus inoculation. Further, the lowest dose that we evaluated, 1 mg/kg/day of (*N*)-MCT, provided significant protection when begun at 2 days post inoculation. When (*N*)-MCT was evaluated in a lethal HSV-2 mouse model, doses as low as 0.01 mg/kg/day of(*N*)-MCT provided protection when initiated within 1 day of intranasal inoculation) while a dose of 25 mg/kg/day significantly reduced virus replication in the brain (Quenelle et al., 2010). Similarly, (*N*)-MCT has been shown to reduce both brain and lung virus titers in mice infected with vaccinia virus, albeit at higher doses, 100–500 mg/kg, than evaluated for HSV (Smee et al., 2007). Safety studies of (*N*)-MCT conducted in mice have revealed no toxicity at doses up to 1000 mg/kg/day (Smee et al., 2007).

The increased efficacy of (*N*)-MCT as compared to ACV may be due differences in potency, bioavailability, and/or the distribution of these two compounds especially CNS penetration. When evaluated for *in vitro* activity, the EC_{50} of (*N*)-MCT against HSV was 0.07 μg/ml compared to 0.3 μg/ml for ACV (Prichard et al., 2006). The pharmacokinetic properties of ACV in rodent models are well characterized (Biron et al., 1982; Sutton and Boyd, 1993) however,

Table 1
Effect of 25 mg/kg/day of (*N*)-MCT when administered beginning 1–2 days post HSV-2 inoculation of newborn guinea pigs.

Group	*N*	Survival (% survival)	# Without symptoms[a] (% without symptoms)
Experiment one			
Control	11	6/11 (55%)	1/11 (9.1%)
ACV 60 mg/kg/day @ 1 dpi × 10 days	11	10/11 (91%)	2/11 (18.2%)
(*N*)-MCT 25 mg/kg @1 dpi × 10 days	11	11/11 (100%)[b]	9/11 (81.8%)[c]
Experiment two			
Control	11	3/11 (27.3%)	0/11 (0%)
ACV 60 mg/kg @ 2 dpi x 10 Days	10	3/10 (30%)	0/10 (0%)
(*N*)-MCT 25 mg/kg @ 2 dpi x 10 Days	11	10/11 (90.9%)[c]	4/11 (36.4%)

dpi: day post inoculation.
[a] As described in methods this includes: vesicular lesions, respiratory, and other general symptoms.
[b] $p < 0.05$ vs. control.
[c] $p < 0.01$ vs. control and ACV.

Table 2
Effect of 1–25 mg/kg/day of *N*-MCT when administered beginning 2–3 days post HSV-2 inoculation of newborn guinea pigs.

Group	*N*	Survival (% survival)	# Without symptoms[d] (% without symptoms)	# With symptoms[a] (% with symptoms)		
				SEM	Respiratory	General symptoms
Control	11	3/11 (27.3%)	0/11 (0%)	11/11 (100%)	11/11 (100%)	6/11 (54.5%)
N-MCT 1 mg/kg @ 2 dpi × 10 days	9	8/9 (88.9%)[b]	1/9 (11.1%)	7/9 (77.8%)	5/9 (55.6%)	1/9 (11.1%)
N-MCT 5 mg/kg @ 2 dpi × 10 days	11	11/11 (80.0% [c]	5/11 (45.5%)[d]	4/11 (36.4%)[b]	2/11 (18.2%)[c]	0/11 (0%)[b]
N-MCT 5 mg/kg @ 3 dpi × 10 days	10	8/10 (80.0%)[d]	0/10 (0%)	8/10 (80%)	9/10 (90%)	3/10 (30%)
N-MCT 25 mg/kg @ 3 dpi × 10 days	6	6/6 (100%)[b]	2/6 (33.3%)	4/6 (66.7%)	3/6 (50%)	0/6 (0%)

dpi: day post inoculation.
[a] As described in methods this includes: vesicular lesions, respiratory, eye disease and general symptoms.
[b] $p \leqslant 0.01$ vs. control.
[c] $p < 0.001$ vs. control.
[d] $p < 0.05$ vs. control.

only one study has been conducted with (*N*)-MCT (Noy et al., 2002). Both ACV and (*N*)-MCT appear to have good selective indices (SI) and little cytotoxicity at the doses we evaluated. Thus, the SI for (*N*)-MCT is > 182 and the cytotoxicity > 100 μg/ml (Prichard et al., 2006). ACV penetration into the brain is impaired in guinea pigs (Myerson and Hsiung, 1983) and humans (package insert; Lycke et al., 2003), perhaps limiting the efficacy of treatment for CNS diseases such as neonatal HSV. The data on (*N*)-MCT concentrations in the brain is limited. In one report (*N*)-MCT levels in the brain were low compared to serum or other organs (Noy et al., 2002). Further pharmacokinetic studies of (*N*)-MCT are warranted for the development of (*N*)-MCT as a potential therapy for HSV infections as well as other herpes viruses such as Epstein-Barr virus (EBV) and Kaposi sarcoma-associated herpesvirus (KSHV) (Prichard et al., 2006; Zhu et al., 2005).

In summary, treatment of newborn guinea pigs with (*N*)-MCT was highly efficacious in protecting the guinea pigs from disease and symptoms of neonatal herpes infection, even when the onset of treatment is delayed. In this regards, (*N*)-MCT was more effective than ACV. Thus, (*N*)-MCT is a promising new drug for neonatal HSV infections in the newborn infant.

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