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Effect of exogenous interferon and an interferon inducer on western equine encephalitis virus disease in a hamster model

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Abstract

Mice are used as models for western equine encephalitis virus (WEEV) infection, but high mortality is generally only seen with intracranial or intranasal challenge, while peripheral inoculation results in approximately 50% mortality and is not dose-dependent. Hamsters were therefore studied as a model for WEEV infection. Hamsters were highly sensitive to intraperitoneal (i.p.) infection with WEEV. Disease progression was rapid, and virus titers in serum, brain, liver, and kidney of infected hamsters peaked between 2 and 4 days post-virus inoculation (dpi). Foci of virus infection were detected in neurons of the cerebral cortex and midbrain. Pre-treatment i.p. with either interferon alfacon-1 (5 μg/kg/day) or with Ampligen® (3.2 mg/kg/day) resulted in complete survival, reduced brain titers, and improved weight gain. This model of WEEV infection in hamsters appears to serve as a suitable model for the evaluation of potential therapeutic agents for the treatment of WEE disease.

Keywords: Western equine encephalitis virus; Alphavirus; Interferon; Infergen; Ampligen®; Antiviral; Hamster; Treatment; Mode

Introduction

Western equine encephalitis virus (WEEV) is a pathogen of human concern that is endemic to the Americas, and periodic outbreaks cause morbidity and mortality in different species including equines and humans (Calisher, 1994). While human outbreaks are infrequent, the case fatality rate may be as high as 15%, and the disease is generally more severe in infants that are less than 1 year old. It is important that therapies be developed for the treatment of this disease (Sidwell and Smee, 2003).

Mice have been used as models for WEEV infection, including antiviral and virulence studies (Bianchi et al., 1993; Hunt and Roehrig, 1985; Monath et al., 1978; Nagata et al., 2005, 2006; Takehara, 1977). Inoculation through peripheral routes, including intraperitoneal (i.p.), intradermal, and subcutaneous, generally results in approximately 50% mortality, and infection through these routes is not dose-dependent as compared with more lethal intracranial and intranasal (i.n.) inoculation (Hardy et al., 1997; Liu et al., 1970). In a mouse pathogenesis study, subcutaneous infection of 1- to 2-day-old mice resulted in high mortality, but pathologic changes were limited to muscle, cartilage, and bone marrow, while infection 3-week-old mice resulted in diffuse meningoencephalitis and changes in heart, lung, kidney, liver, and brown fat (Aguilar, 1970). Previous work has shown that hamsters serve as suitable models for disease caused by WEEV, and high mortality and lesions of encephalitis were observed in adult hamsters inoculated with a virulent strain of WEEV (Zlotnick et al., 1972). Hamsters have been used as models for WEEV infection for immune clearance and virulence studies and are susceptible to WEEV disease (Jahrling, 1976; Jahrling et al., 1983).

Interferon alfacon-1 is a consensus-type interferon that has been efficacious in the clinical treatment of hepatitis C virus (HCV) infections in human patients (Melian and Plosker, 2001; Sjogren et al., 2005; Yasuda and Miyata, 2002), as well as in treatment of various viral diseases and cancer in experimental animal models or cell culture models (Hisaka et al., 2004;

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Morrey et al., 2004a; Takemoto et al., 2004; Yasuda et al., 2000). This cytokine works well in humans as well as in hamsters against different viral diseases (Balan et al., 2006; Gowen et al., 2005; Morrey et al., 2004a). Ampligen® [polyI: polyC12U] is a double-stranded RNA, which elicits an antiviral response at least through the activation of cellular RNase-L and the production of interferon (Anon, 2004). Ampligen® has been shown to have activity against many different viruses and other diseases *in vivo* (Essey et al., 2001; Leyssen et al., 2003; Morrey et al., 2004a; Wild et al., 1996). This compound has also been shown to be efficacious in hamster models of viral disease (Gowen et al., 2005; Morrey et al., 2004a; Smee et al., 1993).

The purposes of these studies were to characterize the WEEV infection of hamsters for use as a model for encephalitic alphavirus infection and to then use the model to evaluate the efficacy of pre-exposure treatment of animals with interferon alfacon-1 and Ampligen®. Such studies with known antiviral agents should establish the utility of the hamster WEEV infection model.

Results

Titration of WEEV in hamsters

Titration experiments revealed that hamsters were highly susceptible to infection with WEEV. Disease progression was rapid and mortality was high in this hamster model. A 100% mortality rate was observed in all i.p. challenge groups with 10-fold dilutions of stock from $10^{6.5}$ down to $10^{3.5}$ $CCID_{50}$, while one animal survived in the lowest ($10^{3.5}$ $CCID_{50}$) dose administered via i.n. inoculation (Table 1, experiment 1). A 60–80% mortality rate occurred in subsequent dilutions down to $10^{1.5}$ $CCID_{50}$ with animals administered by i.p. injection (Table 1, experiment 2). Mean day to death (MDD) appeared to be dose responsive, with shorter times tending to be in animals challenged with higher doses of virus. In the second titration study, weight gain was recorded, and weight change between −24 h and 4 dpi was lower in animals infected with higher doses of WEEV, but this difference in weight was not statistically significant from weight gain in sham-infected controls (Table 1). A $10^{1.5}$ $CCID_{50}$ dose of virus was selected for use in studies characterizing the model and in experiments evaluating antiviral compounds because infection with this dose resulted in 80% mortality.

Table 1
Titration of WEEV infection in hamsters challenged by intraperitoneal or intranasal inoculation

Experiment	Virus dose ($CCID_{50}$)[a]	Inoculation route	Alive/total[b]	MDD±SD[c]	Mean weight change (g)±SD[d]
1	$10^{6.5}$	i.n.	0/5	3.0±0.0	N/D
	$10^{5.5}$	i.n.	0/5	3.4±0.9	N/D
	$10^{4.5}$	i.n.	1/5	3.0±0.0	N/D
	$10^{6.5}$	i.p.	0/5	3.0±0.0	N/D
	$10^{5.5}$	i.p.	0/5	3.3±0.6	N/D
	$10^{4.5}$	i.p.	0/5	4.0±0.0	N/D
2	$10^{3.5}$	i.p.	0/5	4.8±1.1	−1.2±7.0
	$10^{2.5}$	i.p.	2/5	4.7±0.6	3.6±5.9
	$10^{1.5}$	i.p.	1/5	5.0±0.0	5.8±2.6
	Sham-infected	i.p.	3/3	>21±0.0	8.0±4.4

[a] 50% cell culture infectious doses in 0.1 ml inoculation volume.
[b] Number of animals alive on day 21 per total challenged with virus.
[c] Mean day to death±standard deviation.
[d] Mean weight change between −24 h and 4 days post-virus inoculation.

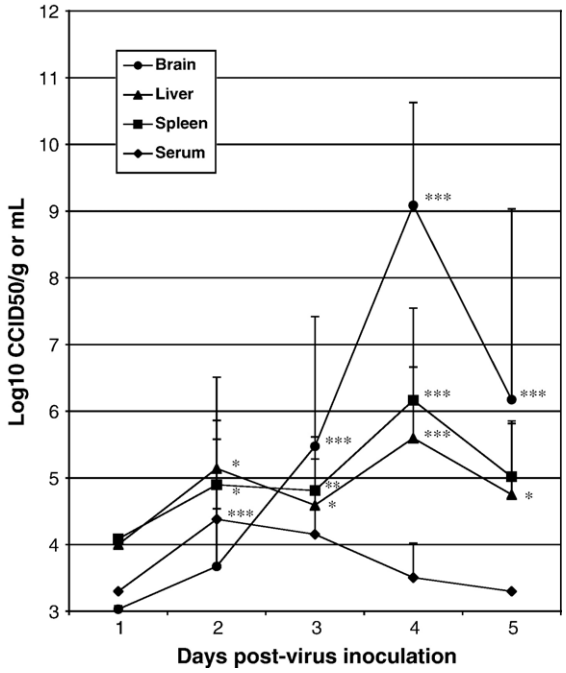

Fig. 1. Time-course of WEEV titer in various tissues from infected hamsters (***$P<0.001$, **$P<0.01$, *$P<0.05$ as compared with sham-infected).

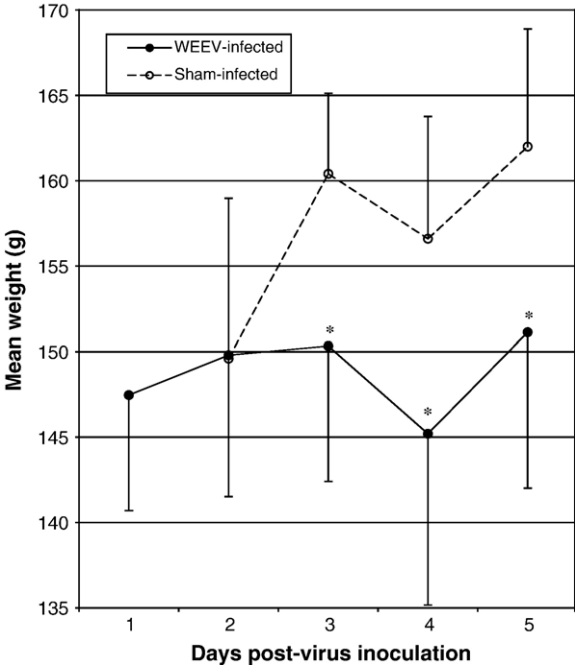

Fig. 2. Weight change of golden Syrian hamsters challenged with WEEV (*$P<0.05$ as compared with sham-infected controls).