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Modeling hamsters for evaluating West Nile virus therapies

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Abstract

A hamster model infected with a New York crow brain isolate of West Nile virus (WNV) was characterized for evaluating potential antiviral therapies. Older hamsters (7–11 weeks old) had a lower mortality of ~50% and more apparent disease signs as compared to >90% mortality in younger hamsters and mice. Disease signs such as limb strength, lacrimation, front limb tremors, somnolence, and deficiences in neurological responses were noted at different times after viral injection. Weight loss was a marker for WNV disease signs, whereas, the ability to climb up an inclined ramp was associated with whether the animals survived the disease or died. Infectious WNV assays performed on tissues from hamsters during development of the infection indicated that viral titers peaked first in plasma, but that titers were eventually highest in kidney tissue. Viral titers achieved maximal levels in brain tissue on 6 dpi, which was 1–2 days before strong neurological signs and death started to occur. Maximal spleen and plasma titers were achieved sooner in young hamsters as compared with older hamsters, which correlated with increased susceptibility. To test the hypothesis that older hamsters would be more sensitive for identifying antiviral effects, Infergen, a consensus human interferon-α highly active against WNV in cell culture, was administered subcutaneously to older and younger hamsters beginning on 2 dpi. The effects of Infergen on weight change, survival, and climbing ability of infected animals were more apparent in older hamsters than in younger hamsters. The use of older hamsters is another WNV-infectious model, in addition to mice, for evaluating potential antiviral therapies.

Keywords: West Nile virus; Model; Antiviral; Hamster; Mouse; Interferon; Infergen

1. Introduction

There has been a heightened interest to identify antiviral agents for the treatment of West Nile virus (WNV) within the United States due to an outbreak in the New York City area in 1999 (CDC, 1999) and a relatively rapid spread across the United States (2002). The virus infection had not been identified within the United States prior to 1999 and is considered to be one more example of an emerging virus due to increased population densities and accessible travel (Zorpette, 2000). This continued expansion of the virus and the possible serious prognosis underscores the need to identify effective antiviral therapies. Suitable animal models are needed for this effort. Laboratory mice have long been used as a model for WNV infection (Haahr, 1968, 1971; Liu et al., 1989; Roehrig et al., 2001; Beasley et al., 2002; Katz et al., 2002). More recently, Xiao et al. (2001) described the use of WNV-infected Syrian golden hamsters as a model to examine encephalitic disease signs, pathology and virology. The report indicated that hamsters had a mortality rate of ~50%, which was more similar to human mortality than with the higher mortality observed in mice (>90%). WNV was also recovered from convalescent hamsters, which was similar to that observed in a monkey model (Pogodina et al., 1983). A unique pathological finding in WNV encephalitis, as compared with other closely related flaviviruses causing encephalitis, is the targeting of Purkinje cells of the cerebellum (Steele et al., 2000). WNV-infected hamsters have neuronal damage and apoptosis of Purkinje cells (Xiao et al., 2001). The purpose of this study was to investigate the utility of the hamster model for preclinical drug discovery for WNV infection.

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2. Materials and methods

2.1. Viruses and animals

Two strains of WNV were used, the Uganda strain B 956 isolated in 1937 (ATCC VR-82, American Type Culture Collection, Manasses, VA) and a New York isolate from homogenized crow brain (NY, CDC 996625, V1 D3 11/10/1999, Robert Lanciotti, CDC, Division of Vector-Borne Infectious Diseases, Ft. Collins, CO). WNV was propagated in African green monkey kidney (Vero 76, ATCC CCL1587) cells using medium 199 with 5% fetal bovine serum (FBS, HyClone, Logan, UT), and 0.18% $NaHCO_3$. Virus stocks were titered in culture using Vero 76 cells using the same medium.

Four- to five-week-old female BALB/c mice, and 4–5- or 7–11-week-old female Syrian golden hamsters were used (Simonsen Laboratories, Gilroy, CA). The animals were challenged subcutaneously with varying titers of 50% cell culture infectious doses (CCID50). Animals were randomly assigned to cages and fed and watered ad libitum.

2.2. Compounds

Infergen™ (lot# P002586 and 202725, interferon alfacon-1, InterMune, Inc., Brisbane, CA) is a second-generation cytokine that was engineered to contain the most frequently occurring amino acids among the non-allelic interferon alpha subtypes. The dose and treatment schedule in animals were based on previously published studies for Infergen (Fish et al., 1986) and unpublished data.

2.3. Cell culture assays

The virus titers in tissues or heparinized plasma were assayed using an infectious cell culture assay (Morrey et al., 2002) where a specific volume of either tissue homogenate or plasma was added to the first tube of a series of dilution tubes. Serial dilutions were made and added to a monolayer of Vero 76 cells in 96-well microplates. Six to seven days later, cytopathic effect (CPE) was used to identify the end-point of infection (Sidwell and Huffman, 1971). Four replicates were used to calculate the infectious doses per milliliter of plasma or gram of tissue (Reed and Muench, 1938).

2.4. Veterinary clinical evaluations

Infected 7–11-week-old hamsters were clinically evaluated over time. Each animal was graded for different disease expressions using a modified neurologic clinical evaluation method of rodents (Irwin, 1968). Each animal was graded daily either until death or 14 days post-viral inoculation (dpi). To minimize bias and potential inter-observer differences, all observations were made by the same individual, who was blinded to the status (infected versus sham-infected) of the groups of animals. The parameters evaluated were as follows: (a) daily body weight, (b) limb weakness: the degree of pelvic elevation while sitting and walking on a flat surface area as a measure of limb weakness, (c) palpebral closure: eye lid closure (normally open fully), (d) tremors: presence or absence, (d) righting reflex: righting after placing the animal on its back (normal values were 1–2 s), (e) hind limb conscious proprioception: sensation and coordination of muscular responses to abnormal limb positioning assessed by placing the dorsal surface of the paw onto a table surface and evaluating the ability and the rapidity with which the animal replaced it to a normal position (normal value was ∼1 s), (f) forelimb grip strength: allowing the animal to grip the wire cage top and subjectively scoring the amount of force required to pull the animal away, (g) abnormal visual placing: evaluated by vertically lowering the animal head-first by its tail to the wire cage top for scoring when and how it placed its forelimbs, (h) lacrimation: presence or absence of ocular discharge (normally absent), (i) salivation: presence or absence of salivation (normally not visible), (j) pinnea: response to stimulation of the ear flap with a pen, (k) cornea reflex: degree of corneal reflex closure after touching the surface, (l) toe pinch reflex: degree of response to mild pain stimuli in the hind feet elicited by pinching a toe with forceps, (m) paralysis: presence or absence, and (n) climbing ability. To perform the climbing assay, hamsters were placed at the lower end of an open-top ramp (10 cm inner width, 15 cm inner height) at a 42° incline. The distance traveled by the hamster in 15 s was recorded. Measurements of uninfected hamsters were taken at night and during the day, after shaking the cage to arouse the hamsters, to determine that both measurements were similar. All hamsters, including those appearing sick, had an apparent motivation to walk up the ramp.

2.5. Infergen treatment

Female hamsters were injected subcutaneously (s.c.) once daily with 5 μg/kg of Infergen or saline placebo in a 0.1 ml volume beginning 2 dpi until 8 dpi. Mean percent weight gain was calculated based on the difference of weights between 3 and 7 dpi, and percent survival was determined up to 12 dpi. Trial #1 had 4–5-week-old hamsters and Trials #2 and #3 had 7–11-week-old hamsters with 10 animals per group except for the saline placebo group of Trial #2, which had 17 animals.

2.6. Statistical evaluation

Differences between mean weight change and viral titer values were analyzed using the *t*-test. Standard deviations were also determined. Wilcoxon and log-rank tests were used to analyze survival data.

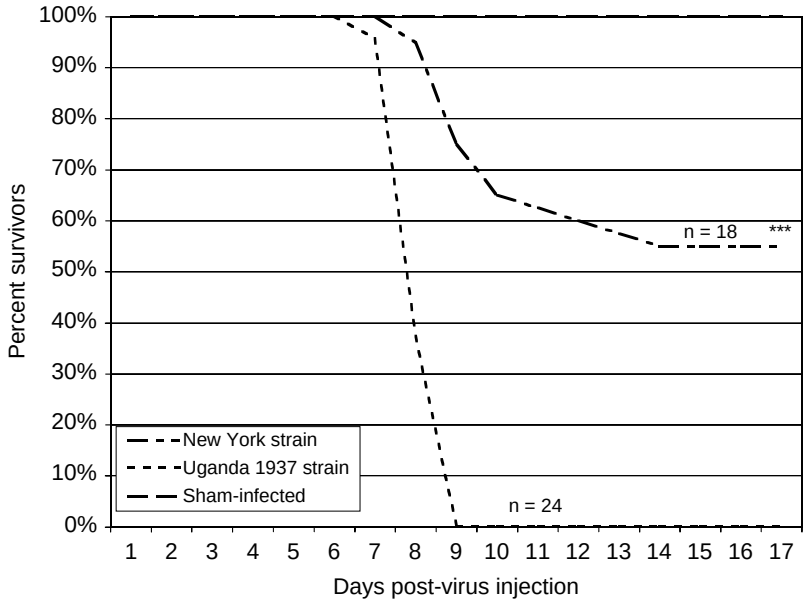

Fig. 1. Comparison of survival between two strains of West Nile virus injected s.c. in 9–11-week-old Syrian golden hamsters. Animals injected s.c. with 10^4 CCID50. *** $P \leq 0.001$.

3. Results

Subcutaneous injection of 9–11-week-old hamsters with the New York isolate of WNV (NY WNV) resulted in a 55% survival, but injection with the Uganda 1937 strain resulted in a 0% survival (Fig. 1). The mean day-to-death (excluding survivors) for New York and Uganda isolates were 9.9 and 8.3 days, respectively. Because of the relevance of the NY WNV as compared with the Uganda strain, studies were continued with the NY WNV.

Subcutaneous injection of the NY WNV in BALB/c mice (Fig. 2) displayed a more consistent dose-responsive survival than intraperitoneal (i.p.) injection, i.e., the survival curve was more related to the viral challenge in s.c. injected-mice as compared to i.p. injections. Injection of hamsters, either s.c. or i.p., did not produce dose-responsive survival using the serial dilution of the virus. Because of the dose-responsiveness of s.c. injection on survival of mice and the closer approximation to natural mosquito infection, s.c. injection was preferred for both mice and hamsters. Older hamsters 7–11 weeks of age, as compared to 4–5-week-old BALB/c mice, have disease signs that are more apparent and have a greater proportion of animals with disease signs (Table 1). For example, 75 and 50% of older hamsters had hind limb paralysis and front limb tremors ($P \leq 0.001$ and $P \leq 0.01$), respectively, as compared with 12% in mice. For this reason we focused on older hamsters to investigate disease signs.

A separate experiment was done in 7–11-week-old hamsters to more thoroughly evaluate other disease signs over the development of infection. The types of abnormalities, the number of animals affected, and the day the signs were first noted in WNV-infected hamsters are summarized in Table 2. Not all animals expressed all signs, nor did they develop signs to the same degree.

The weight change over time in hamsters in response to infection of the New York and Uganda WNV strains was determined (Fig. 3). The sham-infected hamsters gained weight over the course of the experiment, but the animals infected with either isolate dramatically lost weight starting at day 6 dpi. There was little difference in weight change between infected animals that died as compared to animals that

Table 1
Percentage of female hamsters or mice with certain disease signs[a]

	Hind limb paralysis (alive/total)	Diarrhea	Front limb tremor	Ocular discharge
Hamsters (7–11 weeks old)[b]	75% (9/12)***	17% (2/12)	50% (6/12)**	8% (1/12)
Mice (4–5 weeks old)[c]	12% (2/16)	0% (0/16)	6% (1/16)	0% (0/16)

[a] Disease signs were observed until 12 dpi.
[b] Injected s.c. with 10^4 CCID50 of NY WNV.
[c] Injected s.c. with 10^6 CCID50 of NY WNV.
** $P \leq 0.01$.
*** $P \leq 0.001$.

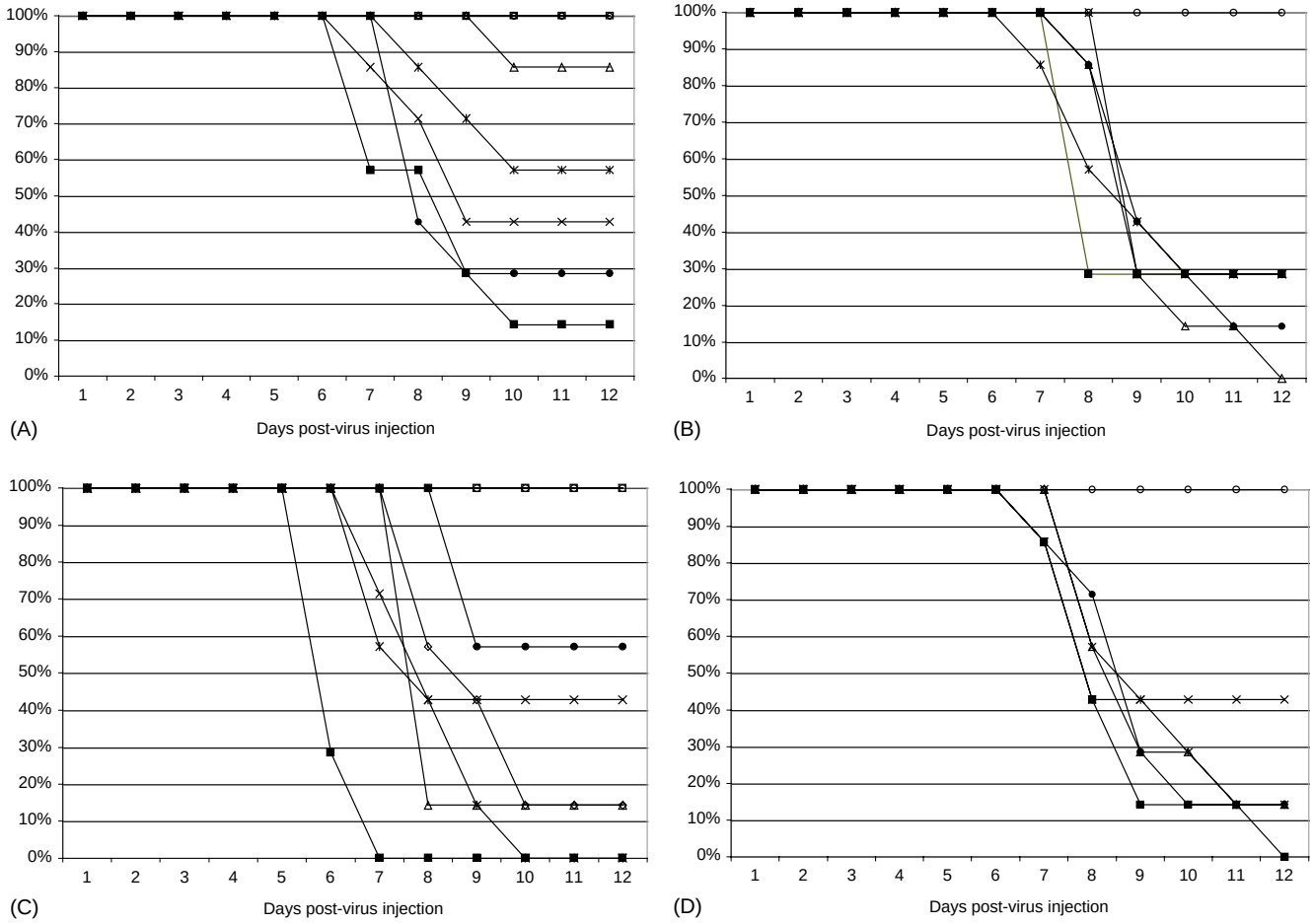

Fig. 2. Survival of animals inoculated with serial dilutions of New York strain of West Nile virus. Seven animals were included in each group. (A) mice, s.c.; (B) mice, i.p.; (C) hamsters, s.c.; (D) hamsters, i.p. (○) Sham-infected; (□) 1 CCID50 of WNV injected; (◇) 10 CCID50; (△) 10^2 CCID50; (×) 10^3 CCID50; (✳) 10^4 CCID50; (●) 10^5 CCID50; (■) 10^6 CCID50.

eventually recovered (data not shown), therefore, weight change was a marker for morbidity, but not mortality.

Climbing ability appeared to be an indicator of eventual WNV-associated death when measured at 7 dpi (Fig. 4). Even though all animals were infected, those animals that survived were able to climb up a 42° inclined ramp on the average of more than twice the distance than those animals that eventually died ($P \leq 0.05$). This marker did not predict

Table 2
Summary of clinical signs in 7–11-week-old hamsters injected s.c. with WNV[a]

Observation	Percent animals affected/total animals (pos/total)[b]	Signs first noted at days post-viral injection (dpi)
Limb weakness	100 (6/6)	3
Palpebral closure	66 (4/6)	10
Tremors	66 (4/6)	9
Decreased righting reflex	33 (2/6)	8
Conscious proprioception	50 (3/6)	8
Decreased grip strength	66 (4/6)	9
Abnormal visual placing	50 (3/6)	9
Lacrimation	83 (5/6)	9
Salivation	0 (0/6)	N/A
Pinna	66 (4/6)	10
Cornea	50 (3/6)	10
Toe pinch	83 (5/6)	8
Paralysis	33 (2/6)	8

[a] Injected s.c. with 10^4 CCID50 of NY WNV.
[b] Number of animals affected/six total animals analyzed.

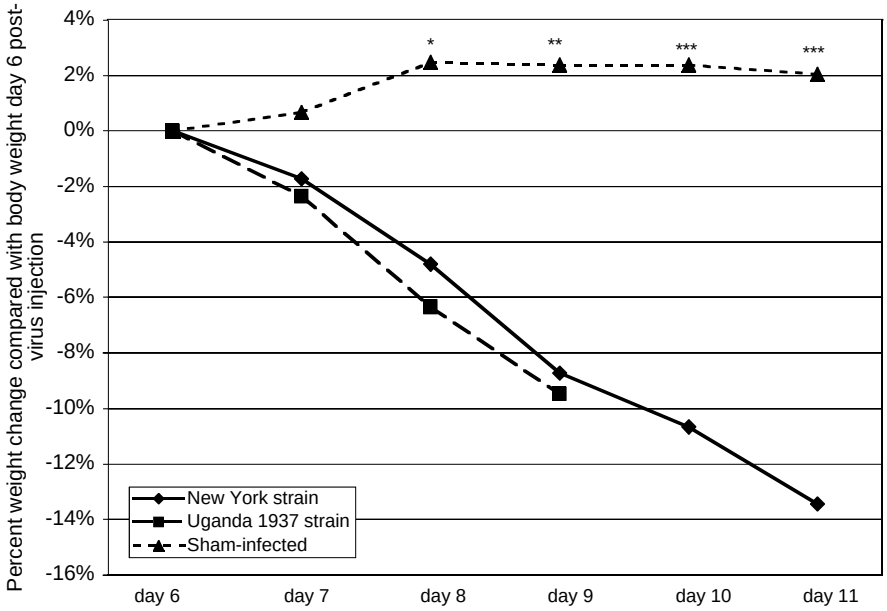

Fig. 3. Percentage weight change in older hamsters (9–11 weeks) injected s.c. with 10^4 CCID50 of either the New York or Uganda strains of West Nile virus. Virus-infected groups had 16 animals each. Sham-injected group had 8 animals each. $^{***}P \leq 0.001$ compared to animals infected with the New York or Uganda WNV isolates.

mortality with certainty, however, because there were some overlap of data points between the survivors and animals that eventually died. The body core temperature parameter did rise ∼1.5 °C in infected animals as compared to uninfected animals (Fig. 5), but there was no difference in body temperatures between survivors and non-survivors (data not shown).

The infectious viral titers in the plasma, spleen, kidney, and brain were determined over a course of time in 7–11- and 4–5-week-old hamsters as a comparison. Larger numbers of animals would be needed to more accurately identify the distribution of virus in organs over time, but trends can be identified with the data. Plasma titers peaked later in older hamsters and earlier in younger hamsters (Table 3). A greater number of younger animals had early detectable infectious virus in the spleen as compared to the kidney or brain, which suggested that the spleen may be an early target organ for virus replication. The highest viral titers were

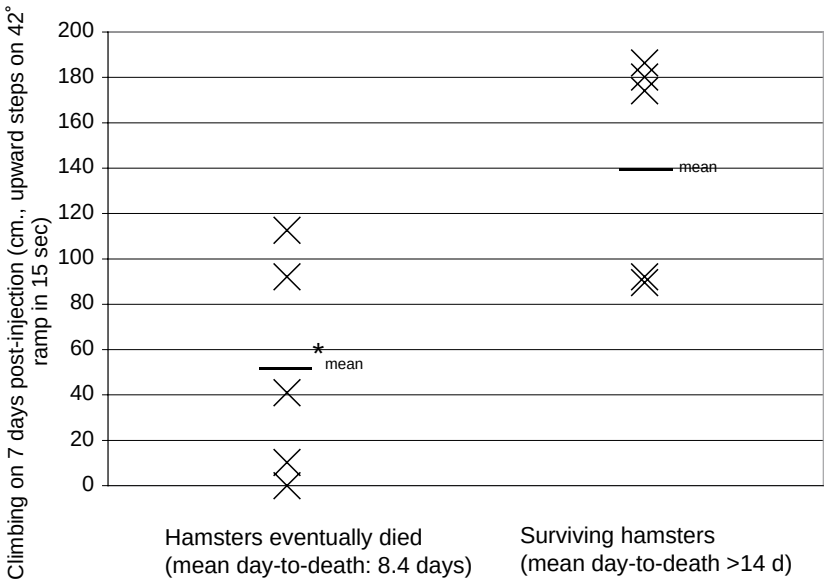

Fig. 4. Correlation of survival with climbing ability 1 day before hamsters started dying (7 dpi) from s.c. injection of 10^4 CCID50 New York WNV. $^{*}P \leq 0.05$ compared to surviving hamsters.

Table 3
Time-course of tissue WNV titers in Syrian golden hamsters[a]

Age (week)	Tissue	Days post-viral injection—viral titers ($\log_{10}$ infectious doses/g tissue or ml plasma ± S.D.									
		1	2	3	4	5	6	7	8	9	10
7–11	Plasma	(1/5)[b]	(1/5)	(5/5) 4.9 ± 0.4	(5/5) 4.1 ± 0.8	(5/5) 3.6 ± 0.9	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)
7–11	Spleen	(0/5)	(4/5) 3.3 ± 1.0	(4/5) 3.4 ± 0.8	(3/5) 4.4 ± 1.5	(4/5) 4.6 ± 1.2	(5/5) 5.0 ± 1.5	(0/5)	(1/5)	(0/5)	(0/5)
7–11	Brain	(0/5)	(1/5)	(1/5)	(1/5)	(1/5)	(5/5) 5.8 ± 1.1	(5/5) 7.3 ± 1.4	(5/5) 7.4 ± 0.7	(5/5) 6.3 ± 1.5	(4/5) 5.7 ± 1.6
7–11	Kidney	(0/5)	(1/5)	(5/5) 5.9 ± 1.2	(5/5) 6.7 ± 0.9	(5/5) 7.4 ± 0.6	(5/5) 8.7 ± 0.8	(5/5) 7.8 ± 0.6	(5/5) 6.7 ± 0.5	(5/5) 6.8 ± 0.4	(5/5) 6.1 ± 0.7
4–5	Plasma	(0/5)	(5/5) 5.9 ± 0.2	(3/3) 5.8 ± 0.3	(3/3) 5.5 ± 0.7	(3/3) 4.3 ± 0.3	(1/3)	(1/3)	(0/2)	–	–
4–5	Spleen	(0/5)	(3/5) 4.4 ± 1.1	(3/3) 6.7 ± 1.3	(3/3) 5.8 ± 1.2	(3/3) 6.4 ± 0.3	(3/3) 5.2 ± 0.4	(2/3) 6.2 ± 3.7	(2/3) 6.2 ± 0.8	–	–
4–5	Brain	(0/5)	(1/5)	(1/3)	(0/3)	(1/3)	(3/3) 5.7 ± 0.3	(3/3) 5.0 ± 1.5	(0/2)	–	–
4–5	Kidney	(0/5)	(1/5)	(3/3) 5.8 ± 0.4	(3/3) 5.0 ± 0.4	(3/3) 6.4 ± 0.2	(3/3) 6.3 ± 0.4	(3/3) 7.6 ± 1.8	(2/3) 6.2 ± 0.8	–	–

[a] Injected s.c. with 10^4 CCID50 NY WNV.
[b] Number of samples with detectable virus/total. Means were not calculated when most samples were below the limits of detection.

present in the kidney even after viremia was gone. This suggested that kidney cells were very permissive for high viral replication. The peak titers in older hamsters occurred later than the corresponding titers in younger hamsters, except for the kidney.

Virus was found at low titers in lower numbers of brains before 5 dpi, which may have been residual virus in the blood of brain homogenates. On day 6, however, virus titers elevated in the brains of older and younger animals, which correlated with the onset of possible neurological signs. On day 7, before mortality started in infected animals, the viral titers in the brain of older hamsters was over 2 $\log_{10}$ greater than in younger hamsters, which inversely correlated with lower mortality in the older hamsters.

To test the hypothesis that older hamsters would be more sensitive for identifying antiviral effects than younger hamsters, because the infected older hamsters were balancing between survival and mortality with a 50% mortality, Infergen, a consensus human interferon-α, was administered s.c. to older and younger hamsters once daily for 7 days beginning on 2 dpi with NY WNV. As expected, the placebo-treated younger hamsters (Trial #1) had 0% survival, whereas, the placebo-treated older hamsters in Trials #2 and #3 had a 50 and 59% survival, respectively (Table 4). The older hamsters displayed weight loss, decreased mortality and decreased climbing ability more than the younger hamsters. In Trials #2 and #3, the older placebo-treated hamsters lost, respectively, 35 and 54% ($P \leq 0.05$) more weight than the Infergen-treated animals, whereas the younger placebo-treated hamsters (Trial #1) lost only 9% less than the Infergen-treated animals. The differences of climbing ability between Infergen- and placebo-treated older animals in Trials #2 and #3 were statistically different ($P \leq 0.01$ and $P \leq 0.05$, respectively), but there was no statistical difference between these groups in the younger hamsters (Table 4). The differences in the percentage survival between the Infergen- and placebo-treated older hamsters for Trials #2 and #3 was 31 and 40% ($P \leq 0.05$), respectively, but the difference in mortality between these groups with the younger hamsters was 20%. Overall, the antiviral effects of Infergen were better detected in older hamsters as compared with younger hamsters. Comprehensive interferon and interferon-inducer studies against WNV in mice and hamsters were reported in a different submitted publication.

4. Discussion

The observation that a Uganda strain of WNV caused 100% mortality in 7–11-week-old hamsters, and that a crow brain New York isolate and a snowy owl New York isolate (strain 385-99) from a different study (Xiao et al., 2001) both caused ∼50% mortality in hamsters indicated that different strains of virus can have different degrees of virulence in this animal model. The Uganda strain is a

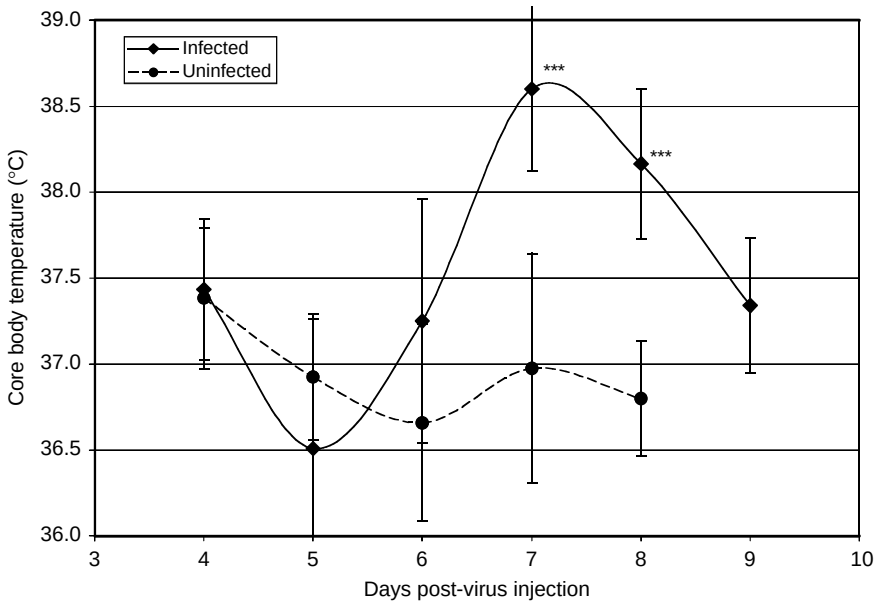

Fig. 5. Core body temperature in 9–11-week-old hamsters injected s.c. with 10^4 CCID50 New York WNV. *** $P \leq 0.001$ compared to uninfected animals.

lineage II typically isolated from Africa, whereas, the New York strains are a lineage I which comprises viruses primarily from the America, Middle East, Europe and some from Africa (Burt et al., 2002). This finding of differences in virulence between WNV strains agrees with a study evaluating the neurovirulence of 19 WNV strains in NIH Swiss outbred mice and in 3–4-week-old hamsters (Beasley et al., 2002). This cited study identified strong neuroinvasive phenotypes from both lineages I and II, and found that the neuroinvasiveness did not correlate with the lineage type. In this previous study (Beasley et al., 2002), various WNV strains were not evaluated in older hamsters (7–11 weeks old), but the results suggested that the virulence of WNV strains, independent of the lineage type, must first be characterized in the Syrian golden hamster before applying this animal model for WNV infection and therapy studies.

The percentage of survival at various dilutions of viral challenge, whether administered s.c. or i.p., did not give a clear challenge dose-responsive curve in hamsters, i.e., the patterns of death were irregular with respect to the cell culture-infectious doses used for the challenge inocula. In mice, however, the higher the viral challenge, the trend for greater mortality was observed. This phenomenon observed in hamsters was previously observed by others in hamsters with WNV (Xiao et al., 2001) and with tick-borne encephalitis virus (Slonim et al., 1966). Consequently, the 50% lethal dose (LD_{50}) in hamsters for these viruses has been difficult to calculate. The biological significance of this observation, if any, is not known, but it does point out a possible disadvantage with the hamster model.

Xiao et al. (2001) previously observed that older hamsters infected with a New York isolate of WNV had "neurological symptoms, including hind limb paralysis, tremors,

Table 4
Comparison of the effect of Infergen treatment[a] in older and younger hamsters infected[b] with a NY strain of WNV

Trial	Hamster age (week)	Compound	Dosage (μg/kg)	Percent survival (alive/total)	Percent weight change[c] ± S.D. (*n*)	Climbing assay[d] (cm/15 s) ± S.D. (*n*)
1	4–5	Infergen	5[e]	20 (2/10)	11 ± 6 (9)	53 ± 48 (7)
1	4–5	Saline	0	0 (0/10)	10 ± 2 (9)	36 ± 33 (7)
2	7–11	Infergen	5	90 (9/10)	−4.3 ± 2.8 (10)	114 ± 38** (10)
2	7–11	Saline	0	59 (10/17)	−6.6 ± 1.7 (15)	68 ± 41 (15)
3	7–11	Infergen	5	90* (9/10)	−2.4 ± 3.2* (15)	56 ± 36* (10)
3	7–11	Saline	0	50 (5/10)	−5.2 ± 3.2 (15)	18 ± 8 (10)

[a] s.c. qd X 7 beginning 2 dpi.
[b] s.c. with 10^4 CCID50 of virus.
[c] Percent change of weight between 3 and 7 dpi.
[d] Distance traveled up a 42° inclined ramp in 15 s on 7 dpi.
[e] No toxicity was identified in toxicity control animals (data not shown).
* $P \leq 0.05$ compared to the respective saline-infected controls.
** $P \leq 0.01$ compared to the respective saline-infected controls.

Table 5
Comparison of West Nile virus human clinical symptoms and signs[a] to those seen in the horse, mouse and hamster

Human subject symptoms (Hubalek and Halouzka, 1999; Weiss et al., 2001)	Horse signs (Porter et al., 2003)[b]	Mouse signs (Haahr, 1968, 1971; Beasley et al., 2002; Katz et al., 2002)[b]	Hamster signs (Xiao et al., 2001)[b]
Fever (influenza-like illness, biphasic, chill)	Fever	Fever	Fever
Transient viremia	Transient viremia	Transient viremia	Transient viremia (1–8 dpi)
Abrupt onset (3–6 dpi)	Abrupt onset (10–11 dpi)	Abrupt onset (5 dpi)	Abrupt onset (3–6 dpi)
Headache often frontal, sore throat	ND	ND	ND
Backpain, myalagia, arthralgia	ND	ND	ND
Muscle weakness, asymmetrical	Muscle weakness, asymmetrical	ND	Muscle weakness, asymmetrical
Conjunctivitis, retrobulbar pain	ND	ND, discharge not present	ND, discharge from eye socket
Maculopapular or roseolar rash	ND	ND	ND
Lymphadenopathy	ND	ND	ND
Anorexia, nasuea, abdominal pain, diarrhea	Anorexia	No diarrhea	Some diarrhea, weight loss
Respiratory symptoms, short breath	ND	ND	ND
Meningitis or encephalitis	Encephalomyelitis	Encephalitis	Encephalomyelitis
Neck stiffness, vomiting	ND	ND	ND
Confusion, disrupted consciousness	Mentation changes	ND	Balance, circling
Acute onset weakness/paralysis and ataxia	Acute onset weakness, ataxia	Acute onset paralysis	Acute onset paralysis
Somnolence	Somnolence	Somnolence, very short term	Somnolence
Tremor in extremities	Muscle fasiculations	Tremors rare	Tremor in extremities
Altered mental status	Mentation changes	ND	ND
Cerebellar abnormality	ND	Brain pathology	Cerebellar pathology
Cranial nerve palsy	Cranial nerve deficits	ND	ND
Coma	Unresponsive	Unresponsiveness short term	Unresponsive
Death (older patients, <0.1%)	Death (30%)	Death (80–100%)	Death (∼50% older hamsters)
Elevated cerebrospinal fluid protein	Elevated CSF protein	ND	ND

ND: not determined.
[a] Not all subjects show all symptoms or signs.
[b] Alignment with human symptoms were subjective and may not correlate exactly.

difficulty in walking, circling, and loss of balance." We determined in this study that the disease signs were more pronounced in older hamsters (7–11 weeks old) as compared with 4–5-week-old BALB/c mice or younger hamsters (data not shown). All disease signs did not result in death, i.e., some animals died with never having paralysis or tremors, and some animals had neurological signs that did not die. One reason for the younger hamsters or mice not displaying the disease signs as readily as older hamsters could be that there was insufficient time for some of the more chronic disease signs to develop, since the course of disease was shorter than in older hamsters. The other possibility was that the disease signs in young animals were more transient, i.e., the signs came and went, before the investigator observed the signs. Hamsters were also about 10-fold larger than mice so detailed observations were easier with hamsters than with mice. Disease signs, therefore, were more thoroughly characterized in the older hamsters.

Weight loss was a strong indicator of WNV morbidity, however, this parameter did not correlate well with animals that eventually died or survived. The ability of the hamsters to climb up a 42° inclined ramp correlated better with the mortality outcome as compared with the weight change parameter. A possible explanation for weight change being a marker for morbidity and the climbing assay being a marker for mortality outcome may be that weight loss was associated with the systemic infection of WNV, regardless of whether central nervous system (CNS) infection occurred. Conversely, the climbing assay may have reflected those animals that had CNS infection with a greater chance for a mortal outcome.

One of the most consistent clinical signs noted in WNV-infected hamsters was muscle weakness. This symptom in infected hamsters ranged from mild cases, which were manifested as a lower body posture while standing or walking, to severe weakness or paresis. The severe weakness or paresis was observed by abnormal sitting postures, and by decreased ability to climb a ramp, or by complete limb paralysis. Because viral titers were not detectable in the brain until approximately 6 dpi, the first observed occurrences of a lowered body posture may be more accurately correlated with a general malaise. However, the persistent and progressive nature of the weakness in eventually 100% of hamsters, and its presentation as both symmetrical and asymmetrical deficits, was comparable to both human (>50%) (Sampson et al., 2000) and equine incidences (94%) (Porter et al., 2003) of WNV infection. Additionally, acute clinical signs of severe weakness and even paralysis observed in hamsters, can also have acute onset in human subjects (Sejvar et al., 2003) and horses (Snook et al., 2001). Muscle weakness and paralysis in the hamster model is an important outcome measurement when evaluating possible new therapies, because muscle paresis or paralysis can be

an outcome in human patients after elimination of viral infection (Solomon and Vaughn, 2002).

Viral titers were determined in plasma, spleen, kidney, and brain over a course of time in 7–11-week-old hamsters having a lower mortality (~50%) as compared with 4–5-week-old hamsters having a higher mortality (~90%) with less apparent disease signs. Virus was detected at earlier time points in younger hamsters as compared with older hamsters, which may have accounted for the higher mortality observed in younger hamsters. It was possible that the rapid expansion of virus did not provide sufficient time for the younger hamsters to mount an effective innate or immune response to the virus, thereby resulting in a higher mortality in the younger hamsters.

The time at which the virus infects the CNS is an important question in relation to disease outcome such as mortality or the onset of debilitating neurological signs. If the neuroinvasiveness of the virus is rapid, the innate or acquired immune system may not have the time to effectively respond to the infection to affect disease outcome. Conversely, if entry of the virus into the CNS is slow, the immune system might respond rapidly enough to diminish disease signs and improve the outcome of the infection. An increase in the number of positively infected brains and the higher level of viral titers in the brains were observed on 6 dpi, which suggests that this was the time at which the virus infected the CNS, however, this will need to be confirmed by immunocytochemistry. The more serious signs of disease, some of which were neurological such as tremors and paralysis, occurred after 6 dpi. The first animals started dying at 7 or 8 dpi. Therefore, it was possible that the virus did infect the CNS at or after 6 dpi and that severe CNS infections eventually killed some of the animals. Nevertheless, infection of the CNS may not be the only determinant for mortality because surviving animals also showed CNS involvement.

The hypothesis that older hamsters were more sensitive for identifying antiviral effects was confirmed with three experiments evaluating the efficacy of Infergen, a consensus human interferon-α. Infergen was administered at 2 dpi instead of at earlier times relative to injection of the virus in order to generate conditions where the antiviral activity would be low and difficult to detect. Infergen demonstrates increased potency when compared to naturally occurring type 1 interferons and is a more potent inhibitor of the hepatitis C virus replication in comparative clinical trials with naturally occurring type 1 interferons (Blatt et al., 1996; Melian and Plosker, 2001). It is also highly active against WNV in Vero cells (data not shown). Infergen is active in hamsters, but not in mice as evidenced by its activity against *herpesvirus* in hamsters. (Fish et al., 1986). Overall, the use of older hamsters better detected the antiviral effects of Infergen as compared with younger hamsters when the disease parameters of percent weight change and climbing ability were used. There was also improvement in survival of Infergen-treated animals as compared to placebo-controls in either older or younger hamsters, but the differences in percent survival was greater when using older as compared with younger hamsters. These differences in survival were not statistically significant, however, so further experiments would be needed to validate this observation. It was important to realize that the interferon regiments, such as 2 days post-viral injection treatments, were anticipated to provide marginal activity so that only the best model would show efficacy. Interferon treatments administered long before the viral injection would have demonstrated efficacy in all models such that the most sensitive model could not be identified.

Comparisons of human, horse, mouse, and hamster disease signs and symptoms are compared in Table 5. All species displayed fever, transient viremia, abrupt onset of disease, somnolence, tremors in extremities, and death. Asymmetrical muscle weakness was detected in all species except mice where the clinical evaluation for this defect was not done. Hamsters were unique in having discharges from the eye socket. Hamsters and human patients also had diarrhea, which was not present in mice or horses. The signs in mice such as rare tremors, no diarrhea, no eye discharge, and short somnolence before death, were not as apparent as in hamsters.

The hamster model, particularly with the use of older hamsters, will provide opportunities to characterize the signs of WNV disease and the effect of therapies and physiological factors on disease outcome. This is possible because of pronounced disease signs and moderate mortality rates where animals can recover or die from the infection. An important issue to be answered will be the identification of clinical markers for outcomes such as neurological motor deficits, sensory or behavioral deficits, and death or survival. Conversely, the older hamster model may not be as useful of a model as younger hamsters or mice if mortality is used as the definitive parameter for identifying efficacy because older animals have a lower mortality as compared with younger animals.

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