Evaluation of the Efficacy Provided by a Recombinant Canarypox-Vectored Equine West Nile Virus Vaccine Against an Experimental West Nile Virus Intrathecal Challenge in Horses

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The efficacy of a recombinant canarypox-vectored equine West Nile Virus (WNV) vaccine was evaluated in a WNV disease model in horses by intrathecal administration of virulent WNV. Ten horses received two doses of a commercial serial of vaccine by the intramuscular route on days 0 and 35, and ten horses were held as unvaccinated controls. Horses were challenged with virulent WNV by intrathecal administration under general anesthesia on day 49. After challenge, eight of ten controls developed clinical signs of encephalomyelitis, whereas one vaccinate exhibited muscle fasciculation in one observation. Fever was present in nine controls and one vaccinate. Histopathology revealed mild to moderate non-suppurative encephalitis in eight controls and one vaccinate. All of the controls and none of the vaccinates had detectable post-challenge WNV viremia. These and the results of previous studies show the efficacy of this vaccine in horses against WNV-induced clinical disease and natural challenge with WNV-infected mosquitoes. Authors’ addresses: Merial Limited, 115 Transtech Drive, Athens, GA 30601 (Siger, Karaca); College of Veterinary Medicine and Biomedical Sciences, Colorado State University, 3801 Rampart Road, Fort Collins, CO 80526 (Bowen); and Merial SAS, 254 rue Marcel Merieux, Lyon 69007, France (Minke); e-mail: Leonardo.Siger@Merial.com. © 2007 AAEP.

1. Introduction

West Nile is an arthropod-born disease caused by a member of the Japanese encephalitis virus complex. The disease is endemic to Africa and the Middle East. In recent years, the disease has emerged in Europe, the Far East, and North America where it has become an important public, veterinary, and wildlife health threat. Most equine infections will result in transient mild disease characterized by fever, but sometimes, the virus causes neurological disease with a fatal outcome.

Histologically, brains of affected horses show a non-suppurative polioencephalitis characterized by infiltration of T lymphocytes and to a lesser degree, by infiltration of macrophages. At present, there is no effective treatment protocol for West Nile Virus (WNV) infection in horses, and control of the disease relies on vaccination and mosquito management. Recently, a disease model that induced clinical disease of WNV in horses by intrathecal administration of virulent WNV into the cisternal space of the atlanto-occipital joint has been reported. This article describes the results and conclusions from a
recent experimental study conducted to assess the efficacy of the canarypox-vectored recombinant WNV vaccine in this clinical disease model.

2. Materials and Methods

Animals

Twenty mixed-breed horses of mixed sex from a commercial herd that were between the ages of 14 and 18 mo were included in this study. None of the horses had detectable antibody against WNV or St. Louis encephalitis virus as shown by plaque-reduction neutralization testing at the beginning of the trial. Horses were housed in a single pasture at a research facility in Montana until day 42 after the initial vaccination; at that time, they were transported to Colorado State University. At this location, horses were housed in a biosecurity level-3 containment building (2–3 horses/room) until the end of the trial. At both sites, horses were fed a diet of pelleted concentrates and maintained in accordance with animal use and care guidelines of the Merial and Colorado State University Institutional Animal Care and Use Committees.

Vaccine Against WNV

A commercial serial of a recombinant canarypox-vectored equine WNV vaccine that contains recombinant canarypox virus expressing WNV prM/E genes derived from a 1999 New York isolate of WNV was used.

Experimental Design

Horses were randomly assigned to one of two groups (ten horses/group). On day 0 and day 35, horses in group 1 received a single dose (1 ml) of recombinant canarypox virus-WNV vaccine by intramuscular administration in the neck. Group 2 horses were not vaccinated and served as controls. On day 49, all horses (ten treated and ten control horses) in the study were sedated with xylazine (1.1 mg/kg body weight, IV) and then anesthetized with ketamine (2.2 mg/kg body weight, IV). Each horse was challenged by intrathecal administration into the cisternal space of the atlanto-occipital joint of 1 ml of virus suspension containing 10⁵ plaque-forming units (pfu) of NY99–4132 WNV originally isolated from an infected crow. After challenge, unused challenge material was submitted to the laboratory for subsequent back titration.

Blood samples for assessment of serum antibodies against WNV were collected from all horses on days 0, 7, 14, 35, 49, 56, 63, and 70. On days 0, 35, and 49, samples were obtained before both vaccination and challenge. Sera were prepared from the blood samples and stored at −80°C until analyzed to determine antibody responses. In addition, blood samples were obtained twice daily from all horses for 7 days after the challenge (days 50–56) and once daily on days 49 (before the challenge), 57–63, and 70 to provide sera for virus isolation. Serum samples were stored frozen at −80°C until virus isolation was performed.

A complete physical examination was performed on each horse twice daily on days 50–60 and once daily on days 48, 49 (before challenge), and 70. Examinations included measurement of rectal temperature and observations of signs of anorexia, depression, muscle fasciculations, lip twitching, head shaking, agitation, hyperactivity, aggression, incessant circling behavior, ataxia, weakness, or reluctance to move; each behavior was recorded as present or absent. Fever was defined as a rectal temperature >102°F (38.8°C).

Horses that developed severe neurological signs as a result of WNV infection were euthanized for humane reasons. At the end of the trial, all remaining horses were humanely euthanized, and a gross and histological evaluation of the brainstem was conducted. Cerebrospinal fluid and two regions of the brainstem (medulla and pons) were collected for WNV-virus isolation, and the remainder of brain was fixed in buffered formalin. A section of brainstem through the pons was stained with haematoxylin and eosin and evaluated histologically by a board-certified veterinary pathologist who was blinded as to the treatment group of the samples. The persons performing laboratory analyses, clinical observations, and gross and histopathological evaluations were blinded to the group assignment.

Viruses Isolation

Serum and cerebrospinal fluid (CSF) samples were assessed for live virus through titration in a plaque assay, as previously described. Briefly, duplicate 0.1-ml samples were inoculated onto Vero-cell monolayers in six-well culture plates and incubated for 1 h at 37°C in an atmosphere containing 5% carbon dioxide. Cells were overlaid with 2 ml of 0.5% agarose in Dulbecco’s modified Eagle medium (without phenol red) that was supplemented with 5% fetal bovine serum and antimicrobials. After 48 h of additional incubation, a second 2-ml overlay of the same solution with 0.004% neutral red was added to the cells. Plaques were counted on days 3, 4, and 5 of incubation, and results were expressed as pfu/ml serum. Brain samples were homogenized in bovine-albumin medium to a concentration of 10% wt/vol, clarified by centrifugation, and then inoculated as described for sera.

Determination of Antibody Responses

Antibody response against WNV was evaluated by use of a plaque-reduction neutralization test. Titration of sera was conducted in blinded fashion as described previously at Colorado State University. Briefly, sera were heat-inactivated at 56°C for 30 min, and serial two-fold dilutions of those sera were mixed with an equal volume (0.1 ml) of a preparation containing 200–300 pfu of the NY99–4132 strain of WNV. After incubation at 4°C for 16–20 h, 0.1 ml of each mixture was laid on Vero-cell mono-
layers in six-well plates and processed as for the plaque assay. Neutralization endpoints were recorded as the highest dilution of serum with which there was a 50% reduction of plaques relative to control wells. A titer of 1:10 or higher was considered to represent a positive antibody response against WNV.

Statistical Analyses
Comparison of the incidence of fever and neurological disease between groups was analyzed using Fisher exact test. The average number of days with clinical signs in the control and vaccinated groups was determined. A repeated-measures mixed-effects analysis of variance with fixed effects of group, day, and group-day interaction was used to compare body temperatures between groups over time. Observations belonging to the same animal were recognized by the model. Pre-challenge average baseline temperature was used as a covariate in the model. Average baseline temperature was calculated using the mean body temperatures on days 48 and 49. All statistical analyses were conducted using SAS software. Statistical significance was based on two-tailed tests of the null hypothesis resulting in p values ≤ 0.05.

3. Results

Antibody Responses
Antibody to WNV was not detected in any pre-immunization serum. Although there was an antibody response starting at 7 days after the first vaccination, none of the vaccinated horses developed an apparent anamnestic response after the first vaccination. At the time of challenge (2 wk after the second vaccination), all ten vaccinated horses had developed detectable antibodies against WNV with a geometric mean titer of 61 (range = 31–118; 95% CI interval and range = 20–160). None of the control horses seroconverted to WNV before the challenge. After the intrathecal challenge, all horses seroconverted, and titers of vaccinated horses increased relative to the day of challenge.

Clinical Signs to the WNV Challenge

Rectal Temperatures
Nine of ten control horses became pyretic (rectal temperature >102.0°F; maximum temperature = 104.2°F) during the post-challenge observation period, whereas only one of ten vaccinated horses developed a single episode of fever (102.2°F) on post-challenge day 13. Fever lasted for an average of 2.1 days (range = 1–3 days) for the control horses that became pyretic. Overall, the rectal temperatures of the vaccinated horses were significantly lower than those of the control animals on post-challenge days 9, 10, 12, and 14 (p < 0.05; F test), and the incidence of fever was significantly higher in the controls versus vaccinates (p = 0.0011; Fisher’s exact test).

Clinical Signs
Eight of ten unvaccinated control horses developed clinical encephalomyelitis characterized by one or more of the following clinical signs: depression, reluctance to move, agitation, weakness, ataxia, tremor, muscle fasciculation, head shaking, and lip twitching. Encephalomyelitis developed primarily between post-challenge days 8 and 13 and had a mean duration of 3.9 days. One control horse became recumbent on post-challenge day 13 and had to be euthanized; another control horse showed persistent signs of WNV disease for 12 days but then recovered. The two horses that did not show any neurological signs developed fever after challenge; duration of the fever was 1 day in one horse and 2 days in the other horse. Of the vaccinated horses, one horse exhibited one of the clinical signs (muscle fasciculation) at one observation point (post-challenge day 12). All vaccinated horses survived until the end of the trial.

The proportion of control horses with neurological disease (eight of ten horses) was significantly higher compared with the vaccinated horses (one of ten horses; p = 0.0055; Fisher’s exact test). The median duration was 3.5 days in the control group and 0 days in the vaccinate group.

Virus Isolation by Plaque Assay
WNV was not isolated from the blood of any vaccinated horse. All ten control horses developed detectable viremia 1–2 days after WNV challenge; peak virus titers were between 10^6.6 and 10^7.9 pfu/ml of serum, and the mean duration was 3.2 days (range = 2–4 days). There was a statistically significant difference in the incidence of viremia between vaccinates and controls after challenge (p < 0.05; Fisher’s exact test).

WNV was not isolated from the brainstem (medulla and pons) or from CSF samples of vaccinates or controls (samples collected at the end of the study).

Post-Mortem Examination
No gross changes were observed in the brain at necropsy.

There was a significantly higher incidence (p = 0.0055; Fisher’s exact test) of brain lesions compatible with encephalitis in the control horses (eight of ten horses) versus the vaccinated horses (one of ten horses). Histopathological changes in the brains of eight control horses included mild (n = 7) to moderate (n = 1) non-suppurative encephalitis characterized by mild to moderate perivascular cuffing with lymphocytes, plasma cells, and multifocal gliosis. Moderate lesions were found in the horse that became recumbent on post-challenge day 13 and had to be euthanized. Two controls did not show any histopathological lesions. One vaccinated horse that had shown clinical signs of WNV disease on post-challenge day 12 showed mild non-suppurative encephalitis of the brain. None of the brains of the
other nine vaccinated horses showed histopathological lesions compatible with encephalitis.

4. Discussion

Merial has developed a canarypox-vectored recombinant WNV vaccine expressing the prM and E genes of WNV. Previously, the efficacy of this vaccine was assessed in response to a challenge with WNV-infected mosquitoes. The results showed that the vaccine provided protection against the development of West Nile viremia as early as 26 days after a single dose. The protective immunity of the product was also proven to last for 1 yr after a primary course of two injections.

The results of the present study clearly show that the vaccine significantly protected horses in a WNV-disease model. Nine of ten vaccinated horses met the criteria for satisfactory clinical protection against a WNV infection that induced neurological disease in eight of ten control horses. Only one vaccinated horse showed mild neurological signs during one observation on one day. Although it has been recently reported that a live chimeric vaccine induced similar levels of protection using a similar challenge model, the recombinant canarypox-vectored equine WNV vaccine is, to the authors’ knowledge, the only vaccine to have shown that it provides protection under both sets of challenge conditions. The clinical signs observed in this experimental model are in line with those seen after natural exposure and were primarily characterized by mentation changes (depression and agitation), muscle fasciculation, tremors, and gait abnormalities with varying degrees of weakness and ataxia. In all but one affected horse, these signs were not progressive; they suggested a diffuse central nervous system disease involving the brain and spinal cord.

Although fever has not been a consistent finding in natural outbreak cases nearly all control horses in the present study developed fever lasting from 1 to 3 days. This difference may be attributed to a low transient fever that may be missed under field conditions. In this study, horses were observed twice daily after the experimental challenge. After challenge, WNV was isolated from the blood of all control horses as early as 24 h after inoculation, which indicates that the blood-brain barrier is disrupted by the inoculation of the virus. Similar findings have been reported in previous studies. Interestingly, both magnitude and duration of viremia were comparable with those obtained after challenge with WNV-infected mosquitoes, however, clinical signs were not observed until 7 days later. Therefore, it takes some time for the virus to induce the changes in the central nervous system that will result in detectable clinical signs. Virus was not isolated from samples collected at the end of the study from the medulla, pons, and CSF. This was not unexpected, because these samples were collected 21 days after the challenge; at that point, the clinical signs in most animals had subsided.

All vaccinated horses developed detectable antibodies to WNV as soon as 7 days after the first vaccination. After the challenge, all control horses seroconverted, whereas vaccinated animals mounted an apparent anamnestic response. The lack of abnormal gross central nervous lesions that was seen in this study is not uncommon; horses naturally exposed to WNV usually have few or no gross lesions.

There was an association between neurological signs and histopathological changes seen in the control horses. However, one control horse had clinical signs but no detectable lesions in the brainstem, and another horse had histopathological lesions in the brainstem but no clinical signs. Similar lesions in the brainstem have been reported in horses after natural exposure and after using this challenge model.

The results of this study and previous studies show significant protection data from a single dose of vaccine and protective immunity lasting for 1 yr after a course of two injections. Therefore, we concluded that this canarypox-vectored WNV vaccine may provide veterinarians with an important tool in preventing WNV infection and disease under high-risk field conditions.

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References and Footnotes


