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Telemetric analysis to detect febrile responses in mice following vaccination with a live-attenuated virus vaccine

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Abstract

Nonhuman primates (NHP) are considered to be the most appropriate model for predicting how humans will respond to many infectious diseases. Due to ethical and monetary concerns associated with the use of NHP, rodent models that are as predictive of responses likely to be seen in human vaccine recipients are warranted. Using implanted telemetry devices, body temperature and activity were monitored in inbred and outbred mouse strains following administration of the live-attenuated vaccine for Venezuelan equine encephalitis virus (VEEV), V3526. Following analysis of individual mouse data, only outbred mouse strains showed changes in diurnal temperature and activity profiles following vaccination. Similar changes were observed following VEEV challenge of vaccinated outbred mice. From these studies, we conclude, outbred mouse strains implanted with telemeters are a sensitive model for predicting responses in humans following vaccination.

Keywords

vaccine; mouse; telemetry

1.0 Introduction

The use of live-attenuated vaccines for prevention of disease in humans and animals has a long, successful history. The ability of the live-attenuated virus vaccines to replicate to a limited extent in the recipient is thought to elicit an immune response reflective of the full repertoire of responses seen in an animal infected by the wild type virus. Live virus vaccines like TC-83,

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the live-attenuated Venezuelan equine encephalitis virus (VEEV) vaccine currently used to vaccinate at-risk personnel, have been produced by a process in which a series of random mutations attenuate virus virulence in a host animal, typically as a result of multiple passages of virus in a cell culture system. In that these mutations can range from a single nucleotide substitution to changes in several amino acids [1] they are susceptible to reversion to the wild type phenotype.

With the ability to manipulate DNA sequences with high precision, it has been predicted that reversion to virulence of vaccine viruses could be prevented by using more complex arrays of attenuating mutations using site directed mutagenesis. A live-attenuated VEEV vaccine candidate, V3526, was developed by Davis *et al.* [2]. V3526 contains a deletion of the PE2 cleavage signal (furin cleavage site) combined with a second-site suppressor mutation in the E1 glycoprotein. These mutations attenuate the virus in its ability to cause overt clinical disease in animals while making reversion practically impossible; however, the virus maintains the ability to replicate and elicit a protective immune response in animals.

Extensive nonclinical testing repeatedly demonstrated that the V3526 vaccine was superior in safety and efficacy when assayed in a variety of rodents [3-7], nonhuman primates (NHP) [8-10] and horses [11] compared to fully virulent strains of VEE IAB virus, enzootic strains of VEEV or TC-83. In safety studies with V3526 conducted in NHP and horses, mild adverse events were observed including temperature elevations, lymphopenia and viral shedding [9-11], but they were typically of short duration and were not present in all animals [6,8,9]. The significance of these adverse events to possible reactions in human recipients was unknown at the time and due to the demonstrated superiority of V3526 over existing VEEV vaccines, the decision was made to proceed to a Phase 1 clinical trial with V3526 in human volunteers. During the clinical trial, fever (Figure 1) and a flu-like syndrome were reported and led to the cessation of further clinical testing of V3526 as a live vaccine candidate [12].

NHP are considered to be the most appropriate model for predicting how humans will respond to VEEV vaccine candidates [13]. The expense associated with the use of NHP is extremely high, as are the space requirements necessary to conduct such studies. Further, the use of NHP when smaller animal models can be used raises ethical concerns. For these reasons, it is desirable to have a rodent model that is as predictive of responses likely to be seen in human vaccine recipients.

Reports of telemetry studies in animals date back to 1966 and at that time because of the size of the implants, the use of telemeters was limited to larger species. Due to the evolution of the technology, an increase in use, particularly in small animals, has been realized [14]. Multiple studies have evaluated continuous monitoring of body temperature and activity in small animals and found the data to be highly reproducible [15-19]. To evaluate the validity of data collected by implanted telemeters, Clements *et al.* [18] found no significant differences between the use of rectal probes and intraperitoneally implanted telemetry devices for collection of temperature data; however, increases in body temperature were reported that appeared to be associated with use of the rectal probe. Collectively, these studies demonstrate the superiority of telemetry over conventional measurement techniques [14]. Since the 1990s, the use of implantable transmitters to measure physiological parameters in conscious, freely moving small animals has become an increasingly prevalent technique in pharmacologic and toxicologic research [20-24]. However, the use of small animal telemetry in studies of infectious disease, particularly vaccine development, has not been widely adopted. The development of telemetric implants for small animals has made possible a more sensitive assessment of murine responses. Subsequent to their implantation in mice, telemetry devices provide an easy, non-invasive method for obtaining measurements of two parameters commonly identified as adverse events, fever and lethargy as evaluated by activity in mice.

The primary goal of this study was to evaluate responses to V3526 vaccination in mice using implanted telemetry devices to determine if the use of telemetry increases the sensitivity of the mouse model for predicting the human response to vaccination. In this study, we also evaluated the degree to which vaccination of mice with V3526 prevents disease following challenge with VEEV IAB Trinidad Donkey strain (VEEV TrD). Since BALB/c mice are an inbred mouse strain and may not reflect the inherent variability in outbred populations (humans and NHP), we also evaluated changes in temperature and activity in two outbred mouse strains, NIH-Swiss and CF-1. This report describes the outcome of these experiments and recommendations for future studies.

2.0 Methods

2.1 Test Material Information

V3526 was produced by Sigma Aldrich Fine Chemicals, Carlsbad, CA. The VEEV TrD virus was produced by Commonwealth Biotechnologies Incorporated, Richmond, VA. Sham-inoculated controls received process control material (PCM), which consists of supernatant from mock infected cultures.

2.2 Animal Information and Environmental Conditions

Female BALB/c, NIH-Swiss and CF-1 mice were purchased from the National Cancer Institute, Fort Detrick, MD. The mice were 6 to 8 weeks old upon arrival and weighed between 15 to 18 g (BALB/c), 18 to 23 g (NIH-Swiss) and 27 to 32 g (CF-1). Mice were group housed in polycarbonate cages with microisolator lids. The room temperature was maintained at $23^{\circ}\text{C} \pm 1^{\circ}\text{C}$ and periods of light and dark alternated on a 12 hour cycle. Animals were provided rodent diet and tap water *ad libitum* throughout the study. Research was conducted at the United States Army Medical Research Institute of Infectious Diseases (USAMRIID) and was in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals, and adhered to principles stated in the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources Commission on Life Sciences, National Research Council, National Academic Press 1996). USAMRIID is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

2.3 Telemetry System

The Data Sciences International (DSI) monitoring system consisted of silicone-coated transmitters, receiver, data exchange matrix and data acquisition system with accompanying software (Dataquest A.R.T. Version 4.1, DSI). The transmitter temperature range had initial accuracy and resolution of 0.01°C and maximal drift of 0.05°C over the warranted 6-month battery life. Activity data is determined by the signal strength between the transmitter and a fixed point in the receiver. Activity data is presented as counts/minute.

2.4 Surgical Implantation of Transmitters

Veterinary technicians, unaware of the study design and dosage groups, randomly selected from group housed mice. Anesthesia was induced by a 0.15 mL injection of mouse KAX anesthetic (60.6 mg/kg Ketamine/ 0.5 mg/kg acepromazine/6.67 mg/kg xylazine). A DSI PhysioTel transmitter (model TA10TA-F20), weighing 3.8 g and occupying 1.75 cm^3 , was aseptically implanted into the peritoneal cavity via a midline or flank incision. For the midline incision, the peritoneum was closed with 5-0 Vicryl with a simple continuous suture pattern. The abdominal muscles were closed with 5-0 Vicryl in a simple continuous suture pattern and the skin was closed using Vetbond. For the flank incision, the peritoneum was closed with 5-0 Vicryl with a single cruciate pattern suture. The muscle was then brought back to proximity

but as it was not incised it had no need to be sutured. The skin incision was closed with Vetbond. The animals were allowed to recover at room temperature on an absorbent pad before being placed individually in cages. Mice recovered from surgery for approximately seven days prior to collection of baseline data.

2.5 Telemetry Data Collection

The data acquisition system was programmed to sample body temperature and physical activity for a 20 second period every 30 minutes. Baseline data was collected for five to seven days. Data collection continued until death or the end of the study.

2.6 Experimental Design

At 8-10 weeks of age, all mice were surgically implanted with telemetry devices. After a seven day recuperative period the mice were transferred to biosafety level-3 containment. After a one-day acclimation period, baseline temperature and activity monitoring was initiated. Mice were inoculated subcutaneously (SC) with either 1×10^4 plaque forming units (pfu) VEEV TrD, 1×10^4 pfu V3526 or PCM (8-10 mice/group) in a 0.5 mL volume. BALB/c and CF-1 mice inoculated with V3526 were challenged SC 28 days later with 1×10^4 pfu VEEV TrD. Telemetry data were collected and body weights were determined daily for 18-21 days post-challenge or until death.

2.7 Determination of Neutralizing Antibody Titers

Virus-neutralizing antibody responses were determined as previously described [25] using VEEV TrD virus as the target in the assay. Sera were serially diluted two-fold and incubated overnight at 4°C with virus. The serum-virus mixtures were further incubated on Vero cell monolayers for one hour at 37°C. The cells were overlaid with 0.6% agarose in Eagle's basal medium with Earle's salts supplemented with 10% fetal bovine serum, 200 IU/mL penicillin, 200 µg/mL streptomycin, 2 mM L-glutamine, and 100 µM non-essential amino acids. Cells were stained with 5% Neutral Red one day later and plaques counted the following day. The endpoint titer was determined to be the highest dilution of serum virus mix with an 80% or greater reduction of the number of plaques observed in control wells.

2.8 Analysis of Telemetry Data

All telemetry data were collected using the DSI DataQuest ART™ software. Upon completion of the study, temperature and activity data were exported from the collection software to Microsoft Excel. To calculate fever hours, the average baseline temperature was determined from the mean temperatures of all implanted mice throughout the seven-day period prior to inoculation. During the course of the study, fever hours were calculated by determining the duration animals had a temperature greater than two standard deviations above the average baseline. Mean fever hours were calculated for each group of animals and ANOVA followed by the Student-Newman-Keuls tests were used to determine statistical significance of the data. To identify differences in activity, the baseline activity data for each strain of mice were averaged to determine average peak activity levels. The average peak activity level was used to evaluate baseline and post-challenge activity levels for individual mice. Activity levels greater than the average peak activity level were considered elevated and the number of elevated activity recordings were tabulated. As the baseline data were evaluated for 5 days prior to inoculation, the post-inoculation period was divided into three, five day segments for the analysis (Days 1-5, Days 6-10 and Days 11-15). The number of pre- and post-inoculation events was compared and Chi-Square analysis was performed to identify statistically significant differences between the baseline and post-inoculation data.

3.0 Results

3.1 Normal Temperature and Activity

Temperature and activity data were collected from age-matched BALB/c, NIH-Swiss and CF-1 mice vaccinated with PCM to evaluate differences between the mouse strains and to identify differences among individual mice within a population. Over a 24 hour period distinct diurnal patterns were observed in all mouse strains with temperatures peaking during nocturnal activity and reaching daily lows during daylight hours. Temperature profiles for all three mouse strains were similar. The average daily range in temperature was 34.5 to 38.2°C for BALB/c mice, 35.8 to 38.5°C for NIH-Swiss mice and 36.1 to 38.3°C for CF-1 mice. The average temperature profile for each mouse strain is shown in Figure 2.

Activity data collected during the baseline period showed sporadic activity throughout any 24 hour period in all mouse strains (Figure 3). NIH-Swiss mice appeared to have lower activity levels than CF-1 and BALB/c mice; however there was no statistically significant difference between the groups as determined by analysis of group averages. Within each mouse strain, no significant differences in activity were observed between individual mice.

3.2 Clinical Observations Post-Inoculation

V3526 was administered SC to mice implanted with telemeters to detect febrile responses following inoculation that may not be detectable by making daily cage-side observations. VEEV TrD was included as a positive control and PCM as a negative control. Following inoculation, mice were observed daily for the onset of signs of disease. In the VEEV TrD-inoculated groups, the first overt sign of disease (decreased grooming) was observed on an average of Day 4 post-inoculation for all mouse strains. Over the next 24 to 72 hours, clinical signs progressed to ruffled fur, lethargy and ultimately death around Day 7. Mice in the V3526 and PCM groups did not present any visible clinical signs of disease.

3.3 Weight

The VEEV TrD-inoculated mice began to show a decrease in weight beginning 2 days post-inoculation, and at 5 and 6 days post-inoculation significant differences in body weights were observed between the treatment groups ($p < 0.01$, ANOVA). As indicated below, this closely follows the onset of fever in VEEV TrD-infected mice. Weight loss continued in the VEEV TrD-inoculated mice, with all mice succumbing by the end of Day 7. In contrast, a gradual increase in weight was observed in the PCM- and V3526-inoculated groups over the course of the study indicative of general good health of the animals (Figure 4).

3.4 Temperature

The diurnal temperature pattern in the VEEV TrD-inoculated BALB/c mice began to deviate from the pattern observed for PCM and V3526-inoculated BALB/c mice approximately 2 days post-inoculation (Figure 5). In PCM and V3526-inoculated BALB/c mice, approximately 12 hours passed between high and low temperatures within a diurnal cycle. However, in VEEV TrD-inoculated BALB/c mice, the duration between the peak temperature recorded on Day 2 and the next recorded trough was approximately 36 hours. In contrast, clinical signs of illness were not detected by daily cage-side observations until 4 days post-inoculation. The fever detected by telemetry on Day 2 was the first indication in this study that the use of telemetric analysis is more sensitive than traditional methods of evaluating onset of disease. In the VEEV TrD-inoculated BALB/c mice, this trough was followed by an increase to maximum recorded temperatures another 36 hours later demonstrating mice undergo a biphasic response to infection similar to the infection kinetics described by Grieder *et al.* [26]. Following peripheral inoculation with VEEV, mice undergo two phases of disease. The first phase is characterized

by virus dissemination through the lymphatic system within 24 hours post-infection and infection of lymphoid and non-lymphoid organs. The second phase begins when the virus invades the central nervous system on Day 2 or 3 post-infection. Mice are not the only species that experience this biphasic response. Biphasic temperature changes have also been reported in equines challenged with VEEV TrD [11]. BALB/c mice did not exhibit changes in temperature profiles following V3526 or PCM inoculation compared to baseline levels.

Similar to BALB/c mice, temperature profiles in NIH-Swiss and CF-1 mice inoculated with VEEV TrD began to deviate from temperature profiles for PCM-treated mice approximately 2 days post-inoculation. Also similar to BALB/c mice, biphasic temperature changes were observed in VEEV TrD-inoculated NIH-Swiss and CF-1 mice; however, the duration between peak temperatures appeared to be more protracted in the outbred strains compared to BALB/c mice. Interestingly, two of the nine NIH-Swiss mice had significantly different temperature profiles between Days 0 and 5 post-inoculation with V3526 compared to baseline temperatures ($p < 0.05$, Student's t-test) and may be indicative of a mild febrile response to vaccination. Temperature profiles in NIH-Swiss mice recorded following PCM inoculation did not differ from baseline profiles. Thirty percent of CF-1 mice had elevated temperatures following inoculation with V3526 whereas four of ten CF-1 mice showed a loss of diurnal rhythm for approximately 9 days following inoculation characterized by a decrease in range of temperatures observed during the baseline period (Figure 6).

The mean fever hours were calculated for each strain of mice following exposure to VEEV TrD, V3526 and PCM. For the BALB/c and NIH-Swiss strains, mean fever hours following VEEV TrD inoculation were significantly higher than V3526- and PCM-inoculated groups ($p < 0.01$, ANOVA with Student-Newman-Keuls post-hoc test) (Figure 7). Mean fever hours for V3526-treated BALB/c mice were not different from PCM-treated mice. Similarly, calculation of mean fever hours for NIH-Swiss mice did not reveal a statistical difference between V3526- and PCM-inoculated mice. This is, however, contradictory to the analysis of temperature profiles from individual animals where approximately 25% of mice had statistically different temperature profiles following inoculation with V3526 suggesting analysis of group means may mask real physiological changes in individual mice. Analysis of CF-1 data by mean fever hours did not detect a significant difference between VEEV TrD- or V3526-inoculated group and the PCM-inoculated group (Figure 7). The size of the error bars in Figure 7 suggest inoculation with V3526 or VEEV TrD leads to a variable fever response in outbred mice and is likely the reason significant differences were not found. Further analysis of the data showed each V3526- and VEEV TrD-inoculated group, could be separated into two statistically different groups: with and without elevated fever hours. The group with elevated mean fever hours, whether inoculated with V3526 or VEEV TrD, was significantly different from PCM-inoculated mice ($p < 0.01$, Student Newman Keuls).

The lack of a significant difference in the mean fever hours between V3526- and PCM-inoculated CF-1 mice when data were not split is similar to reports from NHP studies [9]. Also similar is upon re-analysis of individual NHP data, approximately 50% of V3526-inoculated NHP developed a fever post-inoculation (Figure 8). PCM-inoculated NHP also developed a fever post-exposure; however, there are visible differences in the duration and peak of the fever response from the V3526-inoculated NHP. Particularly notable is the spike ($>3^{\circ}\text{C}$ increase from predicted) in two of the five V3526-inoculated animals shortly after inoculation which is not seen in PCM-inoculated NHP, which developed a low-grade (approximately 1°C elevation from predicted) fever response post-inoculation.

3.5 Activity

Co-incident with onset of fever, decreased activity was observed in VEEV TrD-inoculated mice between 1 and 2 days following inoculation and gradually continued to decline until death

at approximately 7 days (Figure 9) for all mouse strains. The activity of PCM- and V3526-inoculated BALB/c and NIH-Swiss mice was consistent with patterns observed during the baseline period throughout the experiment. Following V3526 vaccination, a trend of increased activity was observed in CF-1 mice. A more in depth analysis of the data revealed 75% of CF-1 mice had increased activity compared to baseline levels following vaccination ($p < 0.01$, Chi Square). In most cases the increase in activity was over a 4 to 5 day period (Figure 10). This increase in activity was not observed in CF-1 mice inoculated with PCM suggesting the increase in activity is related to the V3526 inoculation.

3.6 Temperature and Activity in V3526-Vaccinated and VEEV TrD-Challenged Mice

The amelioration of disease by V3526 vaccination was evaluated in BALB/c and CF-1 mice. Mice were vaccinated with V3526 and challenged SC with VEEV TrD 28 days later. Mice were observed daily for overt signs of illness and temperature and activity were monitored during the baseline, post-vaccination and post-challenge period. Daily observations did not detect any evidence of illness in vaccinated mice following challenge. However, beginning on Day 2 post-challenge, approximately 40% of vaccinated CF-1 mice experienced changes in their diurnal temperature pattern characterized by a decrease in temperature range over a 24 hour period. The range in temperature over a 24 hour period prior to challenge was approximately 35.5 to 38.2°C (range of 2.7°C); whereas post-challenge, the range decreased to approximately 37.0 to 38.2°C (range of 1.2°C), persisting for approximately 5 days (Figure 11). The temperature profiles during the baseline and post-challenge period were significantly different for these mice ($p < 0.05$, Student's t-test). Vaccinated BALB/c mice did not show any changes in temperature following challenge with VEEV TrD suggesting vaccinated CF-1 mice are more sensitive to challenge.

In three V3526-vaccinated CF-1 mice, the activity data collected from Day 1 to 5 post-VEEV TrD challenge were significantly higher compared to baseline activity and corroborate the temperature changes observed in these mice (Figure 12). No changes in activity following VEEV-TrD were found in vaccinated BALB/c mice.

3.7 Neutralizing Antibodies

VEEV neutralizing antibody was detected in all V3526-inoculated mice at 21 days post-inoculation (data not shown). The level of neutralizing antibody titers produced is consistent with studies reported in the literature [4]. Neutralizing antibodies were not found in any of the PCM-treated mice and TrD-treated mice succumbed to infection before blood could be collected.

4.0 Conclusions

In 2005, a Phase 1 clinical trial was conducted to evaluate the immunogenicity and safety of V3526 in human volunteers. The findings from this trial showed robust immune responses in virtually all vaccine recipients [12]. However, a significant number of the vaccine recipients demonstrated mild to moderate adverse events including headache, fever, malaise and sore throat. Nonclinical studies performed prior to the clinical trial revealed lymphopenia and viral shedding in vaccinated NHP; however, febrile responses were not evident [9]. Following the clinical trial, temperature data in NHP were re-evaluated and showed changes in approximately 50% of NHP that may be indicative of a febrile response (Figure 8). It is our belief that the febrile response in PCM-inoculated NHP were due to non-specific inflammatory responses to the components of the PCM while the febrile response to V3526 were due to viral replication and specific inflammatory/immune responses to V3526 replication. Re-evaluation of the NHP data highlighted the importance of examining individual animal responses visually in addition to reporting averaged group data. Mouse data from earlier nonclinical safety and efficacy

testing were not re-evaluated as the original results were based on daily cage-side observations and would not have provided additional insight into whether mice also experienced mild adverse events.

In the current study, mice were implanted with telemeters and vaccinated with V3526 to determine if telemetric data collection would make the mouse more predictive of clinical symptoms in humans, and therefore a useful tool in early stages of vaccine development. These studies are the first indication that vaccination with V3526 produced a mild febrile reaction in mice.

We conducted these studies in BALB/c mice to build upon the analyses conducted during the early stages of nonclinical development of V3526 [3,4,27]. Neither changes in temperature nor changes in activity were detected in BALB/c mice following vaccination with V3526. These results are supported by the general good health of these mice (weight gain, lack of signs of illness) observed in this study and reported in the literature [3,4,27]. In follow-on studies, we evaluated two outbred mouse strains, NIH-Swiss and CF-1, as they may be more predictive of the heterogeneity in the human and NHP populations. In these studies, an increase in temperature post-inoculation with V3526 compared to baseline temperature was observed in approximately 25% of NIH-Swiss mice and 30% of CF-1 mice and a large percentage of CF-1 mice showed increased activity for a few days duration post-vaccination. It is important to note that the percentage of outbred mice with changes in temperature and activity are similar to the percentage of human, NHP and equines with febrile responses following vaccination with V3526. Approximately 30% of humans [12], 50% of NHP and 20% of equines [11] developed a fever of short duration following V3526 vaccination. From this study we conclude the use of telemetry in outbred mouse strains is sufficiently sensitive to detect subtle changes in temperature and activity that may be reflective of the febrile response induced by vaccination in large animal models and humans. However, from these studies it is clear that analysis of changes in individual animals is essential for predicting responses in humans as analysis of group means did not reveal physiological changes in individual mice that were in fact predictive of outcomes in larger species and humans.

To date, evaluation of safety and efficacy in V3526-vaccinated mice has been limited to clinical observations (ruffled fur, hunched back, etc.) and resistance to challenge following immunization [3,4,27]. In the current study, we evaluated temperature and activity in V3526-vaccinated mice following VEEV TrD challenge. All vaccinated mice survived challenge and the daily observations suggested all mice remained healthy during the post-challenge period demonstrating the effectiveness of V3526 in preventing overt disease. However, telemetry data collected from V3526-vaccinated CF-1 mice revealed a remarkable change in daily temperature fluctuations and increased activity following VEEV TrD challenge suggesting that the vaccinated CF-1 mice experience a transient febrile response following challenge.

The results described in this report demonstrate the utility of telemetry in mice, not only for evaluating vaccine safety but also efficacy. Procurement of a telemetry system has a substantial up-front cost, however studies can be planned to take advantage of the long battery life, as telemeters can be reused in multiple, short duration studies. Alternatives to implantable telemeters are available but are often associated with a myriad of disadvantages including the restraint stress induced in animals, aberrant responses due to frequent handling [18] and limited data sets. The effect of limited data sets on the sensitivity of the outbred mouse model described in this report was evaluated by extracting temperature data that would reflect periodic monitoring following V3526 vaccination and following challenge with VEEV TrD. The results of this analysis suggested a minimum of four data collection time points would be required to identify statistically significant changes during the post-exposure period compared to baseline. Although periodic monitoring may have utility, the collection of data would be labor intensive

and data analysis would likely lack the statistical power associated with continuous data collection. Further, periodic monitoring would likely require a longer duration of baseline data collection for establishment of a baseline diurnal pattern as slight variations in baseline temperature readings would likely have a profound effect on detecting differences post-exposure.

Although the current study monitored the responses to V3526 vaccination and VEEV TrD challenge in mice, the use of telemetry holds great promise for advancing our understanding of disease progression and amelioration of disease (vaccination or immunotherapy) in other infectious disease models. In January 2009, the U.S. Food and Drug Administration released draft guidance on addressing efficacy under the Animal Rule [28]. The application of telemetry was suggested as a method to gain information of disease manifestations that may otherwise be difficult to obtain through clinical observations. Importantly, continuous data collection in natural history studies may reveal physiological parameters that are predictors of mortality that could be used as euthanasia criteria in vaccine and therapeutic efficacy studies to prevent unnecessary suffering of animals or to accurately identify triggers of intervention in therapeutic studies.

As with pharmacologic and toxicologic studies, the use of telemetry in studies of infectious diseases has historically been limited to larger species [13,29]. The data presented in this report demonstrate that the addition of telemetric analysis in mice, particularly outbred mice, increases the sensitivity of the mouse model and demonstrates its utility in predicting responses in larger animals and humans. The use of outbred mouse strains appears to be important in predicting responses in heterogeneous populations as individual mice within the inbred mouse strain were found to respond similarly in these studies. Further, analysis of data on an individual basis appears to be critical to detecting febrile responses that may be subtle or of short duration. In conclusion, the data presented in this report demonstrate the utility of telemetry in outbred mice to assess safety and efficacy of a live-attenuated vaccine which may be applicable to testing of vaccines developed on other platforms.

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Figure 1.

Changes in body temperature among V3526 vaccine recipients. The Phase 1 clinical trial evaluated immunogenicity and safety of V3526 in VEEV-naïve healthy adults (18-39 years old). Cohort 1 volunteers (n=10) received 1.25×10^2 pfu V3526, Cohort 2 volunteers (n=10) received 2.5×10^1 pfu V3526 and Cohort 3 volunteers (n=3) received placebo (sterile saline). Temperature was measured daily following administration of V3526. Error bars represent standard error of the mean.

Figure 2.

Average normal temperature profiles collected over a 6 day period from (a) BALB/c, (b) NIH-Swiss and (c) CF-1 mice. Peak temperatures were recorded around 1:00 AM and temperature troughs were recorded approximately 1:00 PM. Data were collected for 20 seconds every 30 minutes. Diurnal temperature profiles were constant over the 21 day observation period therefore only 5 days of temperature recordings are shown.

Figure 3.

Normal average activity profiles collected over a 14 day period from (a) BALB/c, (b) NIH-Swiss and (c) CF-1 mice. Note the overall lower activity levels recorded in NIH-Swiss mice compared to CF-1 and BALB/c mice. Activity peaks on Days 4 and 11 in the NIH-Swiss profile coincide with days when animal cages were changed.

Figure 4.

Data in this graph represents changes in body weight of BALB/c mice only and is relatively similar to changes observed in NIH-Swiss and CF-1 mice (data not shown). All mice in the VEEV TrD-treated group succumbed by Day 7 post-inoculation. Mice in the V3526- and PCM-inoculated groups were weighed for the duration of the study, but only weights out to Day 9 are presented as weights remained unchanged beyond that point. Body weights of VEEV TrD-inoculated mice were statistically different from PCM- and V3526-treated mice on Day 5 and 6 post-exposure. * Body weights statistically different between groups at indicated time points, $p < 0.05$, ANOVA.

Figure 5.

Average temperature profiles for BALB/c inoculated with (a) PCM, (b) V3526 and (c) VEEV TrD. Three days of baseline data are shown followed by 8 days post-inoculation data. PCM- and V3526-inoculated groups maintained their baseline diurnal rhythm throughout the post-inoculation period. In contrast, VEEV TrD-inoculated mice began to deviate from their diurnal temperature pattern at 2 days post-inoculation. Temperatures peaked at 4.5 days post-inoculation and all mice succumbed by Day 7. Similar temperature profiles were observed for NIH-Swiss and CF-1 mice (data not shown).

Figure 6.

Temperature profiles from two CF-1 mice inoculated with V3526. (a) A deviation in the diurnal temperature pattern was observed between Day 14 and 20 whereas in (b) the diurnal pattern was different compared to baseline (not shown) from Day 2 to 10 post-inoculation with V3526. Two additional CF-1 mice showed similar changes.

Figure 7.

Mean fever hours were calculated for BALB/c, NIH-Swiss and CF-1 mice following inoculation with PCM, V3526 or VEEV TrD. Error bars represent standard deviation of the mean. The large error bars represent the variability in responses of the mice to inoculation with V3526 or VEEV TrD. * Statistically different from V3526- and PCM-inoculated groups ($p < 0.01$, ANOVA with Student-Newman-Keuls post-hoc test).

Figure 8.

Temperature profiles for (a) V3526-vaccinated NHP and (b) control-inoculated NHP. Data shown are the residual temperature values for individual NHP obtained by subtracting the predicted body temperature (based on data collected for 14 days pre-vaccination) from the actual body temperatures. Note the sharp peak in the V3526-vaccinated NHP after vaccination compared to the lower but more drawn out fever response in control-inoculated NHP. To achieve better resolution of the graph, only 4 of the 5 NHP data plots are shown.

Figure 9.

Average activity profiles from BALB/c inoculated with (a) PCM, (b) V3526 and (c) VEEV TrD. Three days of baseline data followed by 8 days of post-inoculation data are shown. During the baseline period average activity levels were similar for all mice of the same strain. Changes in activity among VEEV TrD-treated BALB/c mice were noticeable approximately 2 days post-inoculation. Similar changes in average activity profiles were observed for NIH-Swiss and CF-1 mice. Differences in CF-1 mouse activity following V3526-inoculation are shown in Figure 10.

Figure 10.

Increased activity was observed in 75% of CF-1 mice following V3526-inoculation. Increases in activity were typically for 4 to 5 days duration. This activity plot demonstrates changes in activity for one of the CF-1 mice. Note the increase in activity between Day 2 and 6 post-inoculation. This change is statistically significant ($p < 0.01$, Chi-Square) compared to baseline activity.

Figure 11.

Temperature profile from a V3526-vaccinated CF-1 mice following VEEV TrD challenge. Twenty-eight days following vaccination, mice were challenged SC with 1×10^4 pfu VEEV TrD. The range of temperatures recorded during a 24 hour period changed following challenge compared to pre-challenge temperatures. Prior to challenge, temperatures ranged from 35.5 to 38.2°C whereas for about 5 days post-challenge the daily temperature range was less than 1.5°C. In order to show adequate resolution of the profile, only data collected through Day 14 is shown.

Figure 12.

The number of elevated activity points recorded during the baseline and post-challenge period for three V3526-vaccinated CF-1 mice are shown. In all cases, the number of elevated activity points recorded post-challenge were significantly higher than baseline ($p < 0.05$, Chi-Square).