

**International Workshop on Alternative Methods for Human and Veterinary Rabies
Vaccine Testing: State of the Science and Planning the Way Forward**

Poster Session: Wednesday, October 12, 2011

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**Towards the Replacement of NIH Potency Test:
Designing an Immunogenicity Study for Human Rabies Vaccines in OF1 Mice**

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Scope

Replacing the NIH test used to measure the efficacy of both veterinary and human commercial rabies vaccines is a priority in order to refine, reduce and ultimately replace the use of animals in potency testing. One possible alternative is a serological potency assay, as recently shown in an international collaborative study conducted by Paul-Ehrlich-Institut with veterinary rabies vaccines.

Methods

We evaluated the immunogenicity of several human rabies vaccines in dose-ranging studies in adult (9-11 weeks old) OF1 mice. The humoral response following one- and two-intramuscular dose immunization schedules was assessed by protein G ELISA (Bio-Rad Platelia™) and fluorescent antibody virus neutralization (FAVN) test.

Results

Our results showed that an immunization schedule in adult OF1 mice of two intramuscular injections at day 0 and day 21 and blood sampling at day 21 and day 35 allowed the comparison of the humoral responses induced by different human rabies vaccines. FAVN tests detected a sustained humoral response in sera of all mice after a single vaccination with markedly higher titres than those measured by ELISA. Additional results from independent experiments showed that the global variability of results from both FAVN and ELISA were 2-fold lower in our immunization schedule compared with the NIH immunization schedule (3-4 weeks old mice, 2 intraperitoneal immunizations at day 0 and day 7 and blood sampling at day 14).

Conclusions

These results suggest that the measurement of humoral response by FAVN in sera of adult OF1 mice receiving a single intramuscular injection with escalating doses of rabies vaccine could be an appropriate serological method to measure the potency of human rabies vaccines. Such an immunogenicity assay design now has to be evaluated on a larger panel of vaccines in comparison to a NIH standard vaccine and in a large number of trials.

Animal use was carried out in accordance with all applicable animal care and use laws, regulations, and guidelines, and study approved by Ethical Committee.

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Humane Endpoints in Potency Testing of Rabies Vaccine for Human Use in Japan

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Introduction

The potential for introducing humane endpoints in the rabies potency test is an important issue in the quality control for rabies vaccine. Specific weight loss and the presence of specific neurological signs, which are predictive of eventual death in unprotected rabies-infected mice, have been proposed as humane endpoints for rabies vaccine testing. We investigated the potential for introducing humane endpoints in the potency test for human rabies vaccine in Japan.

Materials and Methods

Freeze-dried inactivated rabies vaccine authorized for use in Japan (KAKETSUKEN) was used. Vaccine potency tests were performed according to the standard protocol of the National Institute of Infectious Diseases, which is designed based on the guidelines of the NIH potency test. A total of 450 outbred ddY mice were used in this study. After the inoculation, we observed clinical signs and body weight of mice for 14 days. Clinical signs of infected mice were scored as: Score=1, slow movement, ruffed fur; Score=2, lack of coordination of hind legs; Score=3, whole body paralysis, trembling, coma; and Score=4, death.

Result and Conclusion

Two hundred sixty-four mice died during the observation period, and six mice that were paralyzed on the last day of observation were considered dead from rabies. The average time of death was 10.8 days post inoculation (d.p.i.). Clear neurological signs (Score=3) in mice appeared within an average of 6.4 d.p.i. Body weight of mice that later showed symptoms decreased beginning 4 d.p.i. prior to the onset of symptoms, and significant loss of body weight (>20%) was observed at an average of 6.9 d.p.i. Both indicators found to be equally effective in shortening assessment. These results indicate that there is room for further improvement in the quality control for rabies vaccine in Japan.

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**International Workshop on Alternative Methods to Reduce, Refine, and Replace
the Use of Animals in Veterinary Vaccine Potency and Safety Testing**

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Veterinary vaccines represent an important tool for improving animal and human health by preventing a wide range of infectious diseases in animals and reducing serious zoonotic diseases in people. However, regulatory testing to meet vaccine lot release requirements can require large numbers of animals that may experience unrelieved pain and distress. NICEATM-ICCVAM organized an international workshop in partnership with the European Centre for the Validation of Alternative Methods, the Japanese Center for the Validation of Alternative Methods, and Health Canada to review the state of the science and identify priority activities to advance scientifically sound alternative methods that can further reduce, refine and replace animal use. Nearly 200 scientists from 13 countries participated in the workshop during which they identified relevant knowledge and data gaps and priority research, development, and validation activities to address these gaps. This included identifying opportunities to apply new science and technology to develop improved methods. The highest priority vaccines were Rabies, *Clostridium sp.*, and *Leptospira sp.* vaccines because they require large numbers of animals and involve significant pain and distress. Vaccine challenge testing, which often requires live viruses and bacteria hazardous to laboratory workers, livestock, pets, and wildlife, were also considered high priorities. Collaborations between human and veterinary researchers working on vaccines for the same or similar organisms were recommended to leverage scientific resources and expedite progress. Implementation of the workshop recommendations will likely advance alternative methods for vaccine potency and safety testing to benefit animal welfare while ensuring continued protection of animal and human health.

**World Rabies Day - USDA Center for Veterinary Biologics
Education and Outreach Program**

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As part of the Center for Veterinary Biologics' (CVB) World Rabies Day celebration, we have incorporated an educational outreach project with the surrounding communities. In 2010 the pilot program included Roland/Story Elementary School. Based on positive feedback from the teachers, the program was expanded in 2011 to include West Marshall Elementary School. The program is geared towards rabies prevention through education targeting elementary aged children.

After participation in the lesson, children will: (1) NOT be encouraged to be afraid of pets or wildlife, (2) know to tell their parents or another adult about strange or unfamiliar animals or animal behavior, (3) know NEVER to touch bats, (4) know to NEVER approach wild animals, (5) understand that rabies exists and that it can make people very sick, and (6) understand the importance of vaccination of pets in the prevention of rabies.

The program is aimed at rabies education for grades kindergarten thru fourth. Instructors are given a lesson packet with information to educate students about rabies prevention. Dog bite prevention is also highlighted. After participating in the lesson students are asked to design an art project to reflect what they have learned. Kindergarteners and first graders are asked to color a provided drawing of a bat or a raccoon. The artwork functions as a way to evaluate the effectiveness of our lesson plan and to determine if the correct message was delivered. These posters are collected and become part of CVB's World Rabies Day display.

In 2010 and 2011, this program reached 699 students in 37 classrooms. By educating children we are able to reach whole families; children who have participated in the World Rabies Day events will take home important messages to share with their parents and relatives.

This rabies educational outreach program highlights the importance of education in rabies prevention. The CVB will continue to make effort to expand the number of schools and students involved in this program.

This lesson plan was modeled after information provided by the World Rabies Day website (www.worldrabiesday.org).

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Rabies Vaccine Potency Evaluation Utilizing a Non-Invasive, Inhalational Challenge Method

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Rabies vaccines for veterinary use are essential for safeguarding the public from exposure to rabies virus, as vaccination of domestic animals provides a barrier between humans and the wildlife reservoirs. Ensuring rabies vaccines are potent and effective is paramount in preventing human exposure to rabies virus. The National Institutes of Health (NIH) test, a mouse vaccination-challenge assay, is the most widely used and internationally recommended assay for potency testing of inactivated rabies vaccines and it is currently considered the method of choice. In the NIH test, vaccinated mice are challenged by the intracranial route. The response to the intracranial challenge can be variable, which often results in invalid tests. In addition, the intracranial challenge-exposure raises animal welfare concerns. Modifying the current NIH protocol to administer the rabies virus challenge intranasally may:

- 1) Reduce animal pain and suffering,
- 2) Deliver a more consistent dose of challenge inoculum via a calibrated pipetting device,
- 3) Reduce the number of animals lost to procedure-associated trauma.

The objective of this study was to evaluate the intranasal route of challenge as a modification to the NIH test to reduce animal pain and suffering until harmonized requirements for *in vitro* testing of rabies vaccines are developed. This modification may also improve test precision. Preliminary results confirm that the intranasal route is an effective route of rabies challenge in mice. In addition, a valid challenge may be obtained with a more concentrated inoculum, in comparison to the intracranial method.

All animal testing was performed at the National Centers for Animal Health, an AAALAC accredited facility, under the oversight of the Institutional Animal Care and Use Committee (IACUC).

Key Reference

Rosseels, V et al. 2011. A non-invasive intranasal inoculation technique using isoflurane anesthesia to infect the brain of mice with rabies virus. *J Virological Methods* 173:127-136.

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**International Workshop on Alternative Methods to Reduce, Refine, and Replace
the Use of Animals in Human Vaccine Potency and Safety Testing**

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Few medical interventions have had a greater impact on human health than vaccines. Immunization efforts have resulted in the global eradication of smallpox and the elimination of polio, measles, and rubella in the Americas. Prior to the release of post-licensing production lots of vaccine, regulatory authorities require testing to ensure potency and safety, which can involve large numbers of animals that experience unrelieved pain and distress. NICEATM-ICCVAM organized an international workshop with the European Centre for the Validation of Alternative Methods, the Japanese Center for the Validation of Alternative Methods, and Health Canada to review the state of the science and identify priority activities to advance scientifically sound alternative methods that can reduce, refine and replace animal use in vaccine potency and safety testing. Nearly 200 scientists from 13 countries identified relevant knowledge and data gaps, and identified necessary priority research, development, and validation activities. Diphtheria and tetanus toxoids, pertussis, rabies, anthrax, inactivated polio, and combination vaccines were identified as the highest priority vaccines because they use large numbers of animals and induce significant pain and distress during testing. Research into specific mechanisms of vaccine protection and identifying clinically relevant immunological markers was considered necessary to successfully implement *in vitro* alternatives. Participants agreed that broader acceptance and use of alternative methods would require broader access to information, increased global communication among regulatory authorities, research institutions, and vaccine manufacturers, and harmonization of testing requirements. Implementation of the workshop recommendations is expected to advance alternative methods for vaccine potency and safety testing that will benefit animal welfare while ensuring continued protection of human and animal health.