Use of Animal Models in Rabies Product Development

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Outline

- Introduction to *Rabies virus* pathogenesis
  - Antigenic proteins
- Basic Pathogenesis and transmission
  - Timeline of events *in vivo*
- Experimental rabies
  - Historical background
- Hamster model for post-exposure prophylaxis evaluation
- Closing remarks
Rabies virus pathogenesis
Rabies virus pathogenesis

- Glycoprotein critical for pathogenesis
  - Receptor binding and membrane fusion
- Only external antigen
  - Production of virus neutralizing antibodies
- Epitopes are categorized into regions I-IV & minor site a
  - Antigenic sites II and III are most commonly targeted by natural neutralizing antibodies

History of Animal Models in Rabies Research

- Studies of rabies pathogenesis began in 1804, when Zinke took a small brush and transmitted rabies by swabbing the saliva of a rabid dog onto some fresh wounds he made on the hind leg of a dachshund.
  - That simple procedure showed that the virus could be passed experimentally.

- It was not until Pasteur's monumental studies in the early 1880s that demonstrated that rabies could experimentally be transmitted from animal to animal.
  - Facilitating further studies in pathogenesis, diagnosis, and vaccine production.\textsuperscript{1,2}

\textsuperscript{1} Pasteur L, et al. (1881) l'Academie des Sciences 1881;92:1259-60
\textsuperscript{2} Pasteur L. (1885) l'Academie des Sciences 101:765-72
Most of what we know about the events that take place during rabies infection has been learned from experimental animal models.

<table>
<thead>
<tr>
<th>Year</th>
<th>Species</th>
<th>Treatment</th>
<th>Researchers</th>
</tr>
</thead>
<tbody>
<tr>
<td>1889</td>
<td>Dogs, rabbits</td>
<td>Serum</td>
<td>Babes and Lepp</td>
</tr>
<tr>
<td>1942</td>
<td>Mice</td>
<td>Vaccine</td>
<td>Webster</td>
</tr>
<tr>
<td>1945</td>
<td>Guinea pigs</td>
<td>Vaccine</td>
<td>Habel</td>
</tr>
<tr>
<td>1950</td>
<td>Hamster</td>
<td>None</td>
<td>Koprowski</td>
</tr>
<tr>
<td>1967</td>
<td>Fox</td>
<td>None</td>
<td>Baer</td>
</tr>
<tr>
<td>1971</td>
<td>Monkeys</td>
<td>Serum, vaccine</td>
<td>Sikes et al.</td>
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</table>
Incubation Period Models in Rabies Research

- The most robust animal studies to date examining the events that take place during the incubation period were performed in striped skunks using a Canadian isolate of street rabies virus obtained from skunk salivary glands.

- These studies, performed using reverse transcriptase polymerase chain reaction (RT-PCR) amplification:
  - Demonstrated viral genomic RNA was frequently present in the inoculated muscle (found in four of nine skunks), but not in either spinal ganglia or the spinal cord when skunks were sacrificed 62-64 days post-inoculation.

Incubation Period Models in Rabies Research

- Immunohistochemical studies performed prior to the development of clinical disease showed evidence of infection of extrafusal muscle fibers and occasional fibrocytes at the site of inoculation.

- Although it is unclear, the infection of muscle fibers may be a critical pathogenetic step for the virus to gain access to the peripheral nervous system.

Biological Medical Products and Rabies Animal Models

- WHO recommends in addition to *in vitro* testing of RIG and other products to determine the neutralizing potential some measure of expected efficacy is desirable *in vivo*

- Reproducible animal models should be used for assessing the effectiveness of medical products for *in situ* virus neutralization after infection

- The *in vivo* half-lives of antibody preparations in relevant target tissues should be established for new preparations
  - The levels of antibody required for passive immunization and their duration should be determined, particularly for those based on human monoclonal antibodies

Common Issues with rabies experimental animal models

- Reproducible challenge
  - Variation in mortality by species and strain
  - Outbred vs inbred animals
- Does not replicate human disease
- Non reservoir / target animals
  - Heterologous/homologous variant influenced by species barrier
    - Raccoon variant in raccoons 100% mortality
    - Raccoon variant in hamsters ~30% mortality
- Cost and ethical considerations
  - Companion animals / Non-human primate
Hamster model for evaluation of rabies PEP

- The hamster model has been widely employed as a standardized model to evaluate rabies PEP regimens\(^1,2\)
- In early studies of post-exposure prophylaxis, hamsters were found to be extremely sensitive to rabies virus challenge and demonstrated more reproducible attack rates than other rodent models or NHP models\(^3\)
- High attack rates are observed after intramuscular injection of a large viral inoculum and rabies vaccine is unable to provide complete protection thus facilitating use of the hamster system as a model of severe human exposures to rabies virus\(^4\)

Hamster model for evaluation of rabies PEP

- Based on pathogenesis studies we are able to reproduce:
  - Incubation
  - Clinical signs
  - Failure of vaccine alone to prevent disease for the majority of isolates

Neuronal retrograde viral transport is estimated at 50–100 mm/day (Tsang et al 1991)
Hamster model for evaluation of rabies PEP

- The greatest utility of the hamster model is the ability to evaluate the passive antibody component of PEP regimens.
- When experimentally infected, the mortality rates in hamsters treated with vaccine only PEP approach those of untreated controls (80-100%).
- When passive immunoglobulin is given in addition to vaccine, survival rates of 70-100% are observed.
  - Demonstrates an effective contribution of passive immunoglobulin distinct from rabies vaccine\(^1\)-\(^3\)

Hamster model for evaluation of rabies PEP

- The efficacy of immune serum plus rabies vaccine in the hamster model is similar to that observed in the few human clinical series evaluating the combination of serum and vaccine for rabies PEP.

- The most frequently cited data supporting the critical role of passive immunoglobulin in post-exposure prophylaxis for human rabies is the 1954 WHO field trial of immune serum in Iran.\(^{1-3}\)

- Given the added contribution of passive antibody was only demonstrated in the most severely exposed people, an animal model to evaluate the passive component of PEP should be sufficiently rigorous that vaccine alone is unable to prevent disease.

\(^{1}\) Bahmanyar M. et al. (1976) JAMA 236:2751-2754.
Summary and Conclusions

- A well characterized **animal model is essential** to evaluate any proposed anti-rabies biologic intended for use in PEP scenarios.

- The **global breadth** of *Rabies virus* variants must be considered when evaluating new animal models.

- The **hamster model** shows great potential in addressing many of the confounding factors associated with other **animal models** in rabies PEP evaluation.