ICCVAM/NICEATM/ECVAM Scientific Workshop on Alternative Methods to Refine, Reduce, and Replace the Mouse LD_{50} Assay For Botulinum Toxin Testing (November 13-14, 2006)

Agenda

Abstract

Botulism is a potentially deadly illness. It can be acquired by humans from eating food contaminated with a toxin excreted by the bacterium *Clostridium botulinum*. The toxin causes muscle paralysis due to its action on the nervous system and is the most poisonous substance known. Botulism has been a public health hazard for centuries and today is emerging as a significant bioterrorism threat. Botulinum toxin also has been recently developed into a drug to treat many serious and painful medical conditions that affect the human nervous system.

Currently, the most frequently used method for detecting botulinum toxin in foods or in the environment, or for assessing the potency of the drug, is a test called the mouse LD_{50} assay. This test involves dosing mice with dilutions of the sample being tested and determining the dilution at which 50% of the mice die. The LD_{50} assay has been in use for many years and is currently accepted as the method-of-choice by all U.S. and European regulatory agencies. However, recent scientific and technological advances are providing opportunities for new alternative methods that may be faster and more accurate, and also may refine (less pain and distress), replace, and reduce animal use.

This workshop has been convened to bring together stakeholders and scientists from leading governmental and academic institutions, national and global regulatory authorities, and the animal protection community to review the current state-of-the-science for alternative methods that may reduce, replace, and refine (cause less pain and distress) the use of mice for botulinum toxin testing, and to identify high priority research, development, and validation studies.

Workshop Goals

To review the state-of-the-science and current knowledge of alternative methods that may reduce, replace, and refine (less pain and distress) the use of mice for botulinum toxin testing and identify priorities for research, development, and validation efforts needed to advance the use of alternative methods.

Workshop Objectives

- Review the public health needs for botulinum toxin testing, including the necessity to determine the safety and efficacy of products containing botulinum toxin
- Review the current state-of-the-science and identify knowledge gaps regarding botulinum toxin structural aspects, mechanisms, and modes of action that are important to the development of alternative methods for *in vivo* botulinum toxin tests, and prioritize future research initiatives that would address these knowledge gaps
- Review current development and/or validation status of alternative test methods for *in vivo* botulinum toxin tests and their potential to reduce, refine (less pain and distress), or replace the use of the mouse LD_{50} assay
- Identify alternative methods that should have the highest priority for future development and validation studies to assess potency/toxicity of botulinum toxin

Workshop Agenda and Topics

Day 1 Monday, November 13, 2006

0830 ***Welcome and Introduction of Workshop Goals and Objectives***
- William Stokes, Director, NICEATM
- Len Schechtman, Chair, ICCVAM
- Marlies Halder, ECVAM

Session 1 Overview of Public Health Needs for Botulinum Toxin Testing and Regulatory Requirements

This session will summarize the public health needs for testing and the regulatory requirements in the U.S. and Europe to determine safety and efficacy of products containing the toxin.

Co-Chairs: Abby Jacobs and Jodie Kulpa-Eddy

Food Safety:

0840 ***Overview of Botulinum Toxin and the Incidence and Severity of Botulism***
- Susan Maslanka (Centers for Disease Control and Prevention, US)

This topic will provide a brief overview on botulinum toxin and provide information on the history and global outbreaks of botulism.

0905 ***Current Testing and Practices for Botulinum Prevention in Foods***
- Shashi Sharma (U.S. Food and Drug Administration, CFSAN)

This topic will provide a brief overview of regulations for food safety and testing in the U.S. and Europe.

Drug Safety:

0915 ***Medical Conditions Treated with Botulinum Toxin***
- Mark Hallett (National Institutes of Health, National Institute of Neurological Disorders and Stroke, US)

This topic will provide a brief overview of the clinical applications of botulinum toxin.

0940 ***Current Potency Testing Requirements and Practices for Botulinum Toxin Products***
- Elizabeth Shores (U.S. Food and Drug Administration, CDER)

This topic will provide a brief overview of regulations for drug safety testing in the U.S. and Europe.

Vaccine Potency Testing:

0950 ***Current Testing Requirements and Practices for Botulinum Toxin for Vaccine Potency Testing***
- Jodie Kulpa-Eddy (U.S. Department of Agriculture)

This topic will provide a brief overview of regulations for vaccine safety and potency testing in the U.S. and Europe.

Diagnostic Needs:

1000 ***Current Animal Diagnostic Testing Requirements and Practices for Botulinum Toxin Potency and Detection***

- Tonie Rocke (U.S. Geological Survey, National Wildlife Health Center)

This topic will provide a brief overview of requirements for botulinum toxin detection and testing of environmental samples in the U.S. and Europe.

1010 **Break**

Session 2 **Current Understanding and Knowledge Gaps for Botulinum Toxin**

This session will summarize the current understanding of structural aspects, mechanisms, and modes of action of the botulinum toxin; discuss the aspects of the endopeptidase function that would need to be modeled by alternative test methods; and prioritize research needs to address gaps needed to facilitate the development of alternative test methods.
Co-Chairs: James Keller and Ram Ramabhadran

1030 ***Overview of the Modes and Mechanisms of Action of Botulinum Toxin***

- Dirk Dressler (Rostock University, Germany)

This talk will discuss background information on botulinum toxin mechanisms of action to provide a basis for consideration of information provided in the remainder of this workshop.

1100 ***Pharmacokinetics of Botulinum Toxin***

- Lance Simpson (Thomas Jefferson University, US)

This talk will discuss the absorption, distribution, metabolism and excretion of botulinum toxin *in vivo,* and aspects that will need to be modeled or measured in *in vitro* replacements for the current *in vivo* test.

1130 **Lunch**

1230 ***Essential Characteristics of Potential Test Methods to Replace the Mouse LD_{50} for Botulinum Toxin Potency Testing***

- Eric Johnson (University of Wisconsin, US)

This talk will discuss the criteria for an acceptable replacement for the mouse LD_{50} test method for botulinum toxin potency testing.

1245 ***Overview of U.S. and European Research Initiatives on Botulinum Toxin***

- Lillian Van De Verg (National Institutes of Health, National Institute of Allergy and Infectious Diseases, US)
- Andreas Rummel (Medical School of Hannover, Germany)

This talk will describe current research on botulinum toxin being funded by NIH and in Europe.

1305 **Panel Discussion on the Current Understanding and Knowledge Gaps for Botulinum Toxin (Session 2 Panel)**

- *Moderators:* James Keller and Ram Ramabhadran
- *Panelists:* Dirk Dressler, Mark Hallett, Eric Johnson, Andreas Rummel, Shashi Sharma, Lance Simpson

Session 3 **Potential Replacement of Animal Use for Botulinum Toxin Potency Testing**

This session will provide an overview of alternative *in vitro* models that, if sufficiently validated, could replace the current *in vivo* botulinum toxin test.
Co-Chairs: Susan Maslanka and Shashi Sharma

Session 3A: **Potential Replacement: Endopeptidase Assays**

1405 ***Overview of Endopeptidase Assays***

- Dorothea Sesardic (National Institute for Biological Standards and Control, UK)

This talk will provide an overview of the endopeptidase assays for botulinum toxin detection and potency testing as a proposed alternative for the mouse LD_{50} assay and describe the advantages, limitations, and current validation status of the various detection methods.

1435 **Break**

Session 3B: **Potential Replacement: Cell-Based Assays**

1455 ***Overview of Cell-Based Assays***

- K. Roger Aoki (Allergan, Inc., US)

This talk will provide an overview of frequently used cell-based assays for botulinum toxin detection and potency testing as proposed alternatives for the mouse LD_{50} assay and describe their advantages, limitations, and current validation status.

1525 **Panel Discussion on Potential Replacement of Animal Use for Botulinum Toxin Potency Testing (Session 3 Panel)**

Panel for Session 3A

- *Moderators:* Susan Maslanka and Shashi Sharma
- *Panelists:* John Barr, Frank Gessler, Eric Johnson, Andy Pickett, Ram Ramabhadran, James Schmidt, Dorothea Sesardic, Clifford Shone, Bal Ram Singh

Panel for Session 3B

- *Moderators:* Susan Maslanka and Shashi Sharma
- *Panelists:* Michael Adler, K. Roger Aoki, J. Oliver Dolly, Frank Gessler, Guenter Gross, James Keller, Andreas Rummel, Leonard Smith

1655 **Close of Day 1**

1700 **Poster Session**

Day 2 **Tuesday, November 14, 2006**

Session 4 **Refinement (Less Pain and Distress) of Animal Use for Botulinum Toxin Potency Testing**

This session will provide an overview of alternative methods and approaches that, if sufficiently validated, could reduce or eliminate animal pain and distress associated with the current *in vivo* botulinum toxin test. Three different approaches will be discussed:

- The use of *ex vivo* test models prepared from humanely euthanized animals
- The use of alternate *in vivo* models to measure botulinum activity without lethality
- The use of earlier non-lethal humane endpoints for the current *in vivo* botulinum assay

Co-Chairs: Elizabeth Shores, Leonard Smith, and William Stokes

Session 4A: Refinement: Using *Ex Vivo* Assays to Avoid Pain and Distress in Botulinum Testing

0830 ***Mouse Phrenic Nerve-Hemidiaphragm Assay***

- Andreas Rummel (Medical School of Hannover, Germany)

This talk will provide an overview of the mouse phrenic nerve-hemidiaphragm assay for botulinum toxin detection and potency testing as a proposed alternative for the mouse LD_{50} assay and describe its advantages, limitations, and current validation status.

Session 4B: Refinement: Alternative *In Vivo* Botulinum Assays that Do Not Require Death as an Endpoint

0845 ***Mouse Hind Limb Assay***

- K. Roger Aoki (Allergan, Inc., US)

This talk will provide an overview of the mouse hind limb assay for botulinum toxin detection and potency testing as a proposed alternative for the mouse LD_{50} assay and describe its advantages, limitations, and current validation status.

0900 ***Mouse Abdominal Ptosis Assay***

- Dorothea Sesardic (National Institute for Biological Standards and Control, UK)

This talk will provide an overview of the mouse abdominal ptosis assay for botulinum toxin detection and potency testing as a proposed alternative for the mouse LD_{50} assay and describe its advantages, limitations, and current validation status.

Session 4C: Refinement: Potential Use of Non-Lethal Endpoints in Botulinum LD_{50} Testing to Minimize Pain and Distress

0915 ***Overview of the Physiological Progression of Botulism in Mice***

- Eric Johnson (University of Wisconsin, US)

This talk will describe the progression of the disease of botulism in mice to provide a framework for a consideration of humane endpoints for the LD_{50} assay and alternative *ex vivo* assays.

0925 ***Potential Behavioral and Pharmacological Endpoints Predictive of Mouse Lethality***

- Jerry Calver (Calver Biologics Consulting, Canada)

This talk will describe humane endpoints that could be used to predict lethality in mice used for botulinum testing and the current validation status of each endpoint.

0940 **Break**

0955 **Panel Discussion on Refinement (Less Pain and Distress) of Animal Use for Botulinum Toxin Potency Testing (Sessions 4A, 4B, and 4C Panels)**

- *Moderators:* Leonard Smith and William Stokes
- *Panelists:* Michael Adler, K. Roger Aoki, Jerry Calver, Coenraad Hendriksen, Eric Johnson, James Keller, Andreas Rummel, James Schmidt, Dorothea Sesardic, Martin Stephens

1130 **Lunch**

Session 5 **Reduction of Animal Use For *In Vivo* Botulinum Testing**

This session will discuss strategies to reduce the number of animals used in the current *in vivo* botulinum toxin test.

Co-Chairs: Marlies Halder and Richard McFarland

1230 ***Impact of Sample Size and Toxin Reference Standards on LD_{50} Results***

- Rose Gaines Das (National Institute for Biological Standards and Control, UK)

This talk will provide a statistical consideration of the mouse LD_{50} assay and the effects of decreasing the number of animals tested. This talk also will describe the use of toxin reference standards the mouse LD_{50} assay and the effects on the accuracy, sensitivity, specificity, false positive rate, and false negative rate.

1255 ***Proposed Testing Strategies that Would Reduce Animal Use in Botulinum Toxin Testing***

- Kenneth Clarke (Allergan, Inc., US)

This talk will outline areas that need to be addressed in order to reduce the amount of *in vivo* testing associated with botulinum toxin manufacturing prior to the availability of non-animal replacement assay. Areas include the current protocol used for drug product lot release as well as the potency reference standard program.

1320 **Panel Discussion on Reduction of Animal Use for *In Vivo* Botulinum Testing (Session 5 Panel)**

- *Moderators:* Marlies Halder and Richard McFarland
- *Panelists:* Christopher Bishop, Kenneth Clarke, Rose Gaines Das, Abby Jacobs, Susan Maslanka, Andy Pickett, Tonie Rocke, Dorothea Sesardic, Timothy Terrell

1420 **Break (15 mins)**

Session 6 **Wrap-up of Panel Discussions**
This session will summarize the outcomes from each panel discussion.
Chair: Len Schechtman

1435 ***Summary of Session 2 Discussions: Knowledge Gaps and Research Needs***

- James Keller and Ram Ramabhadran

1450 ***Summary of Session 3 Discussions: Replacement Alternatives***

- Susan Maslanka and Shashi Sharma

1505 ***Summary of Session 4 Discussions: Refinement Alternatives***

- Elizabeth Shores, Leonard Smith and William Stokes

1520 ***Summary of Session 5 Discussions: Reduction Alternatives***

- Marlies Halder and Richard McFarland

1535 **Closing Comments**

1550 **End of Meeting**