PRION WORK
IN
LABORATORY RODENTS

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Introduction

- Transmissible spongiform encephalopathies (TSE) or prion disease are neurodegenerative disease which affect humans and a variety of domestic and wild animal species.

- Prion diseases may present as genetic, infectious, or sporadic disorders, all of which involve modification of the prion protein (PrP).

The Human Prion Diseases

Disease	Abbreviation	Mechanism of Pathogenesis
Kuru		Infection through ritualistic cannibalism
Creutzfeldt-Jakob disease	CJD	Unknown mechanism
Sporadic CJD	sCJD	Unknown mechanism; possibly somatic mutation or spontaneous conversion of PrPc to PrPSc
Variant CJD	vCJD	Infection presumably from consumption of BSE-contaminated cattle products and secondary bloodborne transmission
Familial CJD	fCJD	Germline mutations in PrP gene
Latrogenic CJD	iCJD	Infection from contaminated corneal and dural grafts, pituitary hormone, or neurosurgical equipment
Gerstmann-Sträussler-Scheinker syndrome	GSS	Germline mutations in PrP gene
Fatal familial insomnia	FFI	Germline mutations in PrP gene

The Animal Prion Diseases

Disease	Abbreviation	Natural Host	Mechanism of Pathogenesis
Scrapie		Sheep, goats, mouflon	Infection in genetically susceptible sheep
Bovine spongiform encephalopathy	BSE	Cattle	Infection with prion-contaminated feedstuffs
Chronic wasting disease	CWD	Mule, deer, white-tailed deer, Rocky Mountain elk	Unknown mechanism; possibly from direct animal contact or indirectly from contaminated feed and water sources
Exotic ungulate encephalopathy	EUE	Nyala, greater kudu and oryx	Infection with BSE-contaminated feedstuffs
Feline spongiform encephalopathy	FSE	Domestic and wild cats in captivity	Infection with BSE-contaminated feedstuffs
Transmissible mink encephal-opathy	TME	Mink (farm raised	Infection with prion-contaminated feedstuffs

Introduction

- For nearly five decades with no clue as to the cause, physicians watched patients with a central nervous system degenerative disorder called Creutzfeldt-Jakob disease (CJD) die, often within a few months of its onset.

- Mad Cow disease (BSE) epidemic happened in Britain (more than 180,000 cattle have infected and 4.4 million slaughtered). The BSE crisis led to the European Union banning exports of British beef from1996 to 2006.

Introduction

- Prions are infectious proteins.

- Prions reproduce by recruiting the normal, cellular isoform of the prion protein (PrP^{c}) and stimulating its convention into the disease-causing isoform (PrP^{sc}).

- (PrP^{c}) is rich in α-helical content and has little β–sheet structure, whereas PrP^{sc} has less in α-helical content and a high β–sheet structure.

The Nobel Prize in Physiology or Medicine 1997 was awarded to Stanley B. Prusiner *"for his discovery of Prions - a new biological principle of infection".*

Prion Research

To achieve biological and medical research goals, prion are used in various research experiments (including animal research experiments).

Research goals:

- Basic research: including structural studies, purification, bioassays, and transmission of prions
- Therapeutic studies: including drug and antibody intervention
- Disinfection studies

Prion Animal Research

- PrP was established by biochemical and genetic data leading to knockout, knockin, transgenics, and transplant animal models

- Transgenic mice are produced expressing various species of the prion protein.

- Mutation or substitutions in the prion gene change the expression and translocation of the protein and may change the ***susceptibility*** of the mice to prion infection

- Mice will either be challenged with infectious prions or monitored for spontaneous disease

Prion Animal Experiments

- These experiments raises health and safety concerns to

➢ laboratory personnel

➢ animal facility staff

Challenge of Prion Study

- The transmission modes are not clear
- Prion is resistant to inactivation by heat and chemicals. It is very difficult to decontamination, inactivation and disposal prions
- No effective treatment for prion disease (such as CJD)

How to minimize the bio-hazard risk and work safely with animals administered with prion is a big challenge to biosafety professional.

Goal

- It is a priority for biosafety professional to design and implement a Biosafety program that can minimize biohazard risks of prion and ensures safety working with prion on animals.

Registration

- At UCSF, working with various prions must submit a Biological Use Authorization (BUA) application to the Institutional Biosafety Committee (IBC) and get a approval.

Registration

- Prion use is part of the online BUA application at UCSF

Online BUA application indicates if a study involving:

- Recombinant DNA materials or technology
- Infectious Agents (IA)
- Bloodborne Pathogens (BBP)
- Biological Toxins
- Select Agents
- Animals
- Generate Genetically Modified Animals
- Shipping of biological materials

Registration

For online BUA application, PIs must provide information:

- The purpose using prion
- The experiment procedures
- Used in animals
- Risk assessment
- Method of decontamination, inactivation and disposal
- PPEs use and safety equipments
- Post-exposure procedure

Registration

Select Agent:

- Bovine Spongiform Encephalopathy (BSE)

Federal Select Agent Regulation

- **Select Agent registration with USDA**
- **Select Agent facility**
- **Biosafety plan, Security plan, Emergency Response plan**
- **Training, SRA for users**
- **Inventory (storage, disposal, etc.)**
- **Transfer**

Risk Assessment

Prion is neuropathogenic and can cause human disease. Transmission to human may take years from the initial infection to onset of clinical signs.

Occupational Infections

According to BMBL, no occupational infections have been recorded from working with prions. No increased incidence of CJD has been found among pathologists who encounter cases of the disease post-mortem.

Most of prions have a preference for infection of the homologous species (species barrier), but cross-species infection with a reduced efficiency is also possible.

Risk Assessment

Natural Modes of Infection

- **Ingestion: consumption of infected tissues**
- **Inheritance through the germ line (familial CJD)**
- **It has occurred after transplantation of CJD-infected corneas, received injection of human growth hormones**

Exposure routes:

- **Ingestion**
- **A skin puncture**
- **Contact with mucous membranes (e.g., eyes, nose, mouth)**
- **Contact with non-intact skin**
- **Exposure to aerosols generated during procedures**

Risk Assessment

Aerosols Transmit Prions to Immunocompetent and Immunodeficient Mice

By Johannes Haybaeck, et al.

Department of Pathology, Institute of Neuropathology, University Hospital Zurich, Zurich, Switzerland.

PLos Pathogens 7(1): e1001257, January 2011

Author Summary:

Here we demonstrate that prions can be transmitted through aerosols in mice.

These results suggest that current biosafety guidelines applied in diagnostic and scientific laboratories ought to include prion aerosols as a potential vector for prion infection.

Biosafety Level

BMBL

BSE: BSL2-3

Human prion: BSL2, BSL2*,

All other animal prions: BSL2

UCSF

BSE and human prion: BSL3/ABSL3

Human prion: BSL2*

All other animal prions: BSL2/ABSL2

Safety Training

- **Laboratory Safety for Researchers**
- **Biosafety Level 2**
- **Bloodborne Pathogens training**
- **Animal Biosafety Level 2**
- **Animal Biosafety Level 3**
- **Select Agent training**
- **Laboratory specific training – Laboratory safety manual (prions)**

Personal Protection Equipment

BSL3/ABSL3:

- Jumpsuit
- Double gloves
- Goggles or safety glasses
- Face masks

BSL2/ABSL2

- Lab coats
- Gloves
- Goggles or safety glasses
- Boots (ABSL2)

Exposure/Injury Response Protocol

The UCSF Prion Exposure Protocol

Protocol summary:

- Definition of prion exposure and risk of transmission
- Description and implications of risks
- Exposure follow-up

1. Post-exposure decontamination procedures
   - Eye splash: immediate eye decontamination using an eyewash for 15 min
   - Skin exposure (i.e., contamination of skin, needlestick or laceration): The affected area should be swabbed with ether 5% sodium hypochlorite for 10 min (well tolerate) or 1N NaOH for 5 min if tolerated. When decontaminating with NaOH or sodium hypochlorite, a face shield or eye goggle with mask should be worn for eye protection. The area should be rinsed well afterwards with water to neutralize the base.
2. Call the UCSF exposure hotline

Exposure/Injury Response Protocol

The UCSF Prion Exposure Protocol

Protocol summary:

- Background for clinical providers

- Management of workers potential exposure to prions
  - Employee's responsibilities
  - Supervisor’s responsibility
  - Emergency Department and Employee Health Responsibilities
  - Reporting of exposure by the PI
  - Record-keeping
  - Training for lab personnel

Decontamination Methods

Spill:

- **The surface spill can be wiped up and decontaminated with 1N NaOH (3 times) working from outer rage toward the center.**
- **The surface then be thoroughly rinsed with water**
- **All material that has come in contact with the spill must be disposed as biohazadous waste**

Contaminated equipments:

- **Autoclaved at 132 C for 4.5 hours if the equipment can tolerate it**
- **Using at least 1N NaOH (3 changes, minimum 1 hour) for soak, rinse, or wash objects which cannot be autoclaved**

Waste Management

Liquid waste:

- We employ 1N NaOH final concentration for decontamination of liquid waste because prions are partially resistant to the 1:10 dilution of bleach.

- Liquid waste containing NaOH is held for 24 hours before reducing the pH to below pH 10 and then submitting the waste as pathological waste for incineration.

Waste Management

Solid Waste:

BSL3/ABSL3:

- All solid waste (including animal bedding) will be autoclaved at 132 C, 4.5 hours
- Autoclaved waste will be disposed as pathological waste for incineration

BSL2/BSL2:

All solid waste (including animal bedding) will be disposed as pathological waste for incineration

The Standard Operation Procedure (SOP) for Using Prions in Animals

SOP for inoculation of mice (i.e., intra-cerebral inoculations)

- Two trained animal technicians: a technician in charge of anesthesia and a technician in charge of inoculation
- All inoculations are performed in a biosafety cabinet
- Post-exposure procedure

SOP for animals and tissues transport

Conclusion

- A good biosafety program for using prions
- Safety trainings
- The Standard Operation Procedure for using prions in animals
- The Exposure Protocols for using prions

Will minimizes biohazard risk of prions and ensure safety work with animals




Animal Safety

Procedures for using Infectious Agents in Rodents at ABSL2 Facilities

Procedures for using Lentivirus or Adenovirus in Rodents at the ABSL 2 Viral Shedding Facility

Procedures for using Toxins in Rodents

Procedures for using Carcinogens in Rodents