Cocci is Biosafety Level 3 Pathogen

- Must have animal BSL3 (ABSL3) facilities to work with this pathogen
  - Significant aerosol risk to personnel
  - Proper training
  - Class II biosafety cabinet with extra PPE for protection from aerosols or Class III BSC for intranasal infection
    - Guidelines published in the Biosafety in Microbiological and Biomedical Laboratories, 5th Edition (CDC website)
Mice

• Mice constitute the vast majority of animals used to perform preclinical efficacy studies of antifungal candidates to treat *Coccidioides* infection

• Advantages
  • Very well-established coccidioidomycosis models
  • Small and easy to handle in statistically significant numbers in ABSL3 conditions
  • Wide variety of strains, including genetically engineered for metabolic, immune system defects that mimic human disease conditions

• Drawbacks
  • Pharmacokinetics of drugs may differ significantly from humans
  • *Coccidioides* progresses rapidly in mice
Mice – Routes of administration

- **Pulmonary**  
  - This is the usual way the infection enters the human host  
  - Intranasal or intratracheal in saline suspension  
  - Aerosol of spores in chamber  
- **Intravenous** – rapid, widespread model of dissemination  
- **Intraperitoneal** – dissemination model w/ LFB common readout  
- **Intrathecal** – meningitis, technically challenging  
- **Intracerebral** - meningitis

*High aerosol risk*
Mice – Pulmonary Model

- IN - 50-100 spores in 30-50 µl isotonic saline
  - Under anesthesia
- 4 days to spherule rupture, expansion of infection
  - Some studies treat 48 hrs after pulmonary infection
  - We typically start to treat at 120 hrs, giving time for the infection to establish
    - more similar to human seeking medical care
- 2-3 weeks to moribundity in untreated
  - Know your model to prevent cage death – thin, hunched posture, tachypneic, weak, dehydrated by skin turgor
  - Estimate won’t survive another 24 hrs
Mice – Disseminated Models

• Intravenous
  • ~50 spores - deaths generally after day 12 (Clemons, et. al., 1984)
    • In published studies, treatment is usually instituted within 48 hrs p.i.

• Intraperitoneal
  • Usually requires more arthroconidia to initiate infection by this route
  • Technically easy to perform at BSL3, reduced aerosol risk vs. IN
  • 2-3 week infection model, similar to IN, IV
  • Granulomas of cranial mesentery, spleen, liver, dissemination to lungs
Mice – Disseminated Models

• Intracerebral, intrathecal – meningitis models
  • Clinical signs 6-8 days p.i., deaths usually start by day 8
  • Paresis, paralysis, ataxia, circling, head tilt, seizures, obtundation
    • Need to be evaluated twice daily for animal welfare reasons once onset of clinical signs
  • Generally institute treatment within 48 hrs p.i.
• Assess fungal burdens or survival
  • Assess lungs and spleens, not just brain and spinal cord
Assessment of Mouse Models

• Survival (moribundity)

• Organ fungal burdens
  • Common assessment, may be the primary measure
  • Eradication vs. reduction in colony-forming units (cfu)
  • Quantitate cfu by 10-fold serial dilutions of homogenized tissues
    • Usually lung and spleen
    • Can qualitatively assess dissemination by incubating whole organs on plates

• Clinical signs, body weight
  • Body weight is a good indicator of progression of infection even before other signs become visible
Rabbits

• Clemons and colleagues developed a reliable model of coccidioidal meningitis/arteritis in rabbits
  • Cisternal infection
  • Size of animal allows some serial cisternal sampling of CSF
  • Post mortem analysis can include histopathology, fungal burden of spinal cord, brain, CSF parameters
    • Meningitis/arteritis in rabbits appears to be a good model for humans

• Understand drug PK in this species
  • Less utilized than mice in development

• Increased cost of animals, labor, housing
  • Potentially fewer animals, less robust statistics

• Facilities need to be able to manage larger model at ABSL3
Nonhuman Primates

• Experimental - possible but really costly
  • Intratracheal infection with suspension of arthroconidia
    • Recommend a nebulizer after working out a dog model (Soffler, Bosco-Lauth et al. 2012)
  • Current trends seem to support that most drugs at this stage would be implemented in some kind of human trial

• There could be opportunities to treat naturally infected NHPs in primate centers within the endemic area
  • Administration and monitoring offer challenges
Naturally Infected Dogs

• This is an interesting preclinical assessment of drug efficacy
  • In endemic region such as southern and central AZ, very high case load and it is not difficult to enroll cases

• Possible to assess pulmonary disease improvement with 30-60 days of treatment
  • Radiographs, serology, CBC-serum chemistries
  • Dog owners are both grateful and dedicated
    • <10% dropout rate in 2 studies I have done, compliance is very good

• Drawbacks
  • Cost, time to perform, statistically significant numbers, may not drive your development
    • Often get descriptive data from such a study

• Potential advantages
  • Naturally occurring disease in model that is already sick, like the human that presents for medical care
  • Oral administration using forms of drug that would be used in people
  • Might be preclinical PK/toxicology in this species so you know how to dose them
Summary

• The mouse model is the work horse of pre-clinical testing of antifungal drug candidates
  • Small and cost-effective animal for studies that have to be run at BSL3
• The rabbit is a well-developed meningitis/arteritis model that can be used
  • Costs, facilities and technical expertise with the procedures are a drawback
• Larger animal models, both naturally infected and laboratory induced, exist
  • Weigh benefits and costs of these