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Humane Endpoints

- Death as an endpoint is not acceptable
- Earliest time point in the experiment where the animal can be humanely killed without loss of data
Standards

• Animal Welfare Act
• Public Health Service Policy on Humane Care and Use of Laboratory Animals
• US Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training
Standards

• Appropriate species, quality and minimum number to obtain valid results
• Avoid or minimize discomfort, distress, and pain consistent with sound research design
• Procedures that cause pain or distress in human beings may cause pain or distress in other animals
Standards

- Painful or distressful procedures performed with appropriate sedation, analgesia, or anesthesia
- Prohibition on using paralytics in awake animals
- Animals experiencing severe or chronic pain or distress that cannot be relieved will be euthanized
• ‘The central problem, ..., is ... how humanity can be promoted without prejudice to scientific and medical aims’
  – Direct inhumanity – unavoidable consequence e.g., toxicity testing, proof of virulence
  – Contingent inhumanity – incidental and inadvertent e.g., husbandry, post operative recovery, inter current disease
Humane Endpoints Concepts

- Good science and humane animal care are complimentary
- Must know normal to recognize abnormal
- Meaningful, easily recognizable and adhered to
- Minimizes animal pain, distress and discomfort
- Permit achievement of scientific objectives
Recognizing Pain and Distress

• Pain is potential or actual tissue damage that is perceived by the central nervous system characterized by avoidance or need to avoid.

• Distress is an aversive state in which the animal is unable to cope resulting in maladaptive behavior and/or abnormal physiological responses that if continued for prolonged periods will be detrimental to the animal’s health.
Stress

• Stress is an aversive state that elicits an adaptive response that ultimately enriches or primes the animal to become better adapted to its environment.

• Stress is good, distress is bad.
Recognizing Pain and Distress

- Change in body weight, external physical appearance, clinical signs, unprovoked behavior, and behavioral responses to external stimuli.
- Clinical Scores to promote continuity of good care. Morton. ILAR J 41:80-86;2000
  - Emaciated, under-conditioned, well-conditioned, over-conditioned, obese (BC 1-5).
Cancer Research

- Disorganized cell growth, invasion and tissue destruction
- Animals used to study mechanistic processes and therapeutic approaches
- Potential to cause pain, distress and discomfort due to the tumor and drugs used.
Monitoring Tumor Development

- Tumor growth
- Tumor growth delay
- Tumor regression
- Clonigenic survival of tumor cells
- Moribundity or death
β-Human Chorionic Gonadotropin (HCG) as a biomarker of tumor progression

- Simple, cost effective, widely applicable
- β-HCG transfected tumor cells injected intraperitoneally (IP)
- Urinary and serum HCG highly correlated
- Urinary HCG highly correlated with tumor size

Infectious Disease Models

- Animals are used to study mechanisms of [infectious] disease development, assess treatment and prevention strategies.
- Pain, distress and discomfort can result from the infectious agent, failure of therapy or prevention, or toxicity of test materials.
Guinea Pig Sepsis Model

• Evaluation of antimicrobial agents and host pathogen interactions in infected neutropenic guinea pigs.

• Surrogate markers for mortality in *Pseudomonas aeruginosa* sepsis.

• Potential surrogate markers
  – ruffled fur, respiratory distress, diarrhea, hunched posture, lethargy, abnormal neurologic movements (twitching, paralysis of a limb), inapparentance >48 h, inability to ambulate, inability of a supine animal to stand, weight loss, daily water or food consumption
  – Checked q4h during day, q8h at night

Louie et al LAS 46:617-623; 1997
Guinea Pig Sepsis Model

- 100% of animals unable to ambulate or rise from supine position died
- Animals unable to rise from supine position died in 1-8 h
- Animals unable to ambulate died in 4-40 h
- 59-69% of ambulatory animals were found dead at next observation – rapidly progressive
Mouse Sepsis Model 1

- Characterize a murine model of staphylococcal enterotoxic shock
  - Staphylococcal enterotoxins (SE) ⇒ SEA-SEJ
- Avoid death as an endpoint
- Telemetry devices with temperature and activity sensors implanted intra-abdominally

Vlach et al. Comparative Medicine 50:160-166;2000
Mouse Sepsis Model 1

- SEB and/or lipopolysaccharide (LPS) administered intraperitoneally
- Body temperature and physical activity decreased after SEB and LPS or LPS alone but not SEB only
- Body temperature early predictor of mortality
  - At $<23.4^\circ\text{C}$, 86% of all mice died
  - At $<23.4^\circ\text{C}$, 96% of mice given SEB plus LPS died
  - Rectal temperature not recommended due to potential for rectal injury
Mouse Sepsis Model 2

- Evaluation vaccines for efficacy against gram negative sepsis
- Avoid death as an endpoint
- Galactosamine sensitized mice given LPS and monitored every 4 h.

Mouse Sepsis Model 2

- Body surface temperature measured with infrared camera.
- Weight, posture, coat, inability to ambulate, loss of consciousness
- Loss of ability to ambulate predicted death 2-22 h.
Cardiomyopathy Surgical Model

• Left ventricular (LV) function following myocardial infarction (MI) is the most important predictor of adverse prognosis.
• Coronary artery occlusion is a model of MI induction in large animals but has high mortality in rats.
• Cryothermal injury of the left ventricle resulted in a highly reproducible impairment of LV function and reduced mortality.

Huwer et al Comparative Medicine 50: 385-390; 2000
Imaging Modalities

- Imaging Modalities e.g. Positron Emission Tommography, Magnetic Resonance Imaging, Ultrasound as aids to achieving humane endpoints will be discussed in detail later by Dr. Gabrielson.

- Progression of disease in individual animals non invasively resulting in fewer animals used and less pain and distress.
Conclusions

• The preceding examples demonstrate that we can use biomarkers, experimental data, physiological and behavioral parameters to develop humane endpoints that benefit individual scientific studies.

• Humane endpoints are study specific.

• Additional resources are available in the subsequent slides.
Conclusions

• Good science necessitates humane animal care
• Must know normal to recognize abnormal
• Meaningful, easily recognizable and adhered to
• Minimizes animal pain, distress and discomfort
• Permits achievement of scientific objectives
Study Questions

• What are the principal considerations in developing humane endpoints in an infectious disease study?

• An investigator would like to develop a surgical model of heart failure. What would be your advice to the investigator regarding when to terminate the study?

• An investigator would like to use death as an endpoint in an infectious disease study, what you suggest to the individual?
Resources

- [http://www.jhu.edu/animalcare/](http://www.jhu.edu/animalcare/)
- [http://www.jhu.edu/animalcare/committee3.html](http://www.jhu.edu/animalcare/committee3.html)
- IACUC Training and Compliance staff
- Comparative Medicine
- JHU-mousers
Resources

• ALTWEB
  – Humane Endpoints
  – http://altweb.jhsph.edu/topics/humane-endpoints.htm

• University of California Center for Animal Alternatives (UCCAA)
Resources

• Humane Endpoints for Animals Used in Biomedical Research and Testing. ILAR Journal 41(2); 2000

• Impact of Noninvasive Technology on Animal Research. ILAR Journal 42(3); 2001

• Advanced Physiological Monitoring in Rodents. ILAR Journal 43(3); 2002
Resources

• Experimental Design and Statistics in Biomedical Research. ILAR Journal 43 (4); 2002.

• Bioethics of Laboratory Animal Research. ILAR Journal 40(1); 1999.
Resources


• http://www.oecd.org/ehs/
Resources