Endpoints in Animal Study Proposals

Introduction

Experimental studies may involve procedures that cause clinical symptoms or morbidity in animals. Death as an endpoint is not acceptable. Beyond the undue discomfort and distress experienced, potentially valuable scientific information may be lost as well when consideration for appropriate endpoints are not incorporated within the Animal Study Proposal. The Animal Care and Use Committee must consider the selection of the most appropriate endpoint(s). This requires careful consideration of the scientific requirements of the study, the expected and possible adverse effects the research animals may experience (pain, distress, illness, etc.), the most likely time course and progression of those adverse effects, and the earliest most predictive indicators of present or impending adverse effects. The effective use of endpoints requires that properly qualified individuals perform both general and study specific observations of the research animals at appropriate time points. Optimally, studies are terminated when animals begin to exhibit clinical signs of disease if this endpoint is compatible with meeting the research objectives. Often experiments are designed to capture various stages of induced disease. In these cases, it is pertinent for the Animal Study Proposal to correlate disease progression with clinical signs and appropriate endpoints. Such endpoints are preferable to death or moribundity since they minimize pain and distress. Efforts must be made to minimize pain and distress experienced by animals used in research.

Morbidity

Animal Study Proposals that include morbidity as an endpoint or that include animal procedures that have the potential to cause adverse sequelae should address the following:

1. Criteria that establish when the endpoint has been reached.

    A. There are several examples in the literature that might be considered, including:

        1. Evaluation of five aspects of an animal's condition as described by Morton and Griffiths. These are body weight, physical appearance, measurable clinical signs, unprovoked behavior and response to external stimuli.
        2. Clinical observations used in cancer research and toxicological studies as described by Montgomery.[1]

    B. The clinical signs, depending on severity and duration, that may constitute an endpoint include, but are not limited to:

        20% weight loss *(emaciated appearance; rapid weight loss over two to four days; or progressive weight loss over a few weeks)*
        Diarrhea, if debilitating
        Progressive dermatitis
        Rough hair coat, hunched posture, lethargy or persistent recumbency
        Coughing, labored breathing, nasal discharge
        Jaundice and/or anemia
        Neurological signs (frequent seizure activity, paralysis)
        Bleeding from any orifice

Self-induced trauma
Any condition interfering with eating or drinking (e.g. difficulty with ambulation)

Excessive or prolonged hyperthermia or hypothermia
Proptosed eye
Prolapses that progress to tissue damage and necrosis

C. Additional signs in neoplasia studies that may constitute an endpoint include, but are not limited to:

1. Primary tumor size (20mm in diameter for a mouse and 40 mm in diameter for a rat). Formulas for calculating tumor size can be found in the literature (see tumor size references). Justification to exceed this size restriction must be approved by the NCI-Frederick ACUC in advance.
2. Tumors that ulcerate, become necrotic or infected.
3. Palpation of tumor induces a pain response (vocalization, flinching, withdrawal).
4. Tumors that interfere with the ability to eat, drink, or ambulate.

D. Any animal found unexpectedly to be moribund, cachectic, or unable to obtain food or water.

2. A plan should be implemented for monitoring the animals both before and after a change in any of the above aspects, providing care if appropriate, and increasing the level of monitoring. Monitoring or clinical care on weekends and holidays may require involvement of the investigative staff to supplement that provided by the animal care and veterinary staff.
3. For tumor regression studies, careful attention should be paid to any animal exhibiting an ulcerated and/or necrotic tumor. To deter cannibalization, any animal exhibiting an ulcerated or necrotic tumor should be separated immediately and singly housed until tumor regression is complete. A watch card should be placed on each individual cage containing a mouse with an open tumor, recording the date of the tumor opening on the card. Personnel are responsible for ensuring adherence to (a) ACUC approved regression timelines; (b) endpoints as described in the animal study proposal; (c) the ACUC Guidelines for Experimental Neoplasia; and (d) the ACUC Guidelines for Endpoints in animal study proposals (i.e., euthanizing the mouse if the tumor becomes infected, interferes with ambulation/eating/drinking, or the mouse becomes otherwise debilitated).
4. Personnel should be identified who are responsible for evaluation, record keeping, notification of the investigator and/or veterinarian and persons responsible for euthanasia. Checklists/ score sheets/intervention charts may be helpful in ensuring appropriate observations are made, consistently interpreted, and properly documented.
5. An *Animal Disposition Authorization Form* should be completed by the investigator and kept on file in the animal facility for the duration of the study.

Moribundity

While it is preferable to use the earliest endpoints compatible with the scientific requirements of each study, there are studies that require moribundity as an endpoint. The moribund condition is defined as a clinically irreversible condition leading inevitably to death. In these studies, animals are permitted to become moribund, as a result of experimental procedures. In some cases, pain-relieving measures are not used because such measures may compromise experimental integrity. Examples of research proposals that may have moribundity as an endpoint include: infectious disease studies, drug and toxicity studies, and cancer research. The following

guidelines are suggested to assist the Animal Care and Use Committee in reviewing proposals with moribundity as endpoints.

Animal Study Proposals utilizing moribundity as an endpoint should contain the following information:

1. The scientific rationale for moribundity as an endpoint, including:

A. What alternatives were considered, why morbidity as an endpoint cannot be used, and how alternatives will be used whenever possible.
B. Why pain-relieving measures cannot be utilized.
C. Number of animals to be used and why this is the minimal number of animals required.
D. Expected duration of moribundity prior to euthanasia.

2. A plan for the following animal care and monitoring procedures:

A. Animals involved in experiments that may lead to moribundity will be monitored at least daily by personnel experienced in recognizing signs of morbidity (illness, injury, or abnormal behavior) for at least the following: abnormal posture, rough hair coat, perianal soiling, neurologic signs, hypothermia, exudate around eyes and/ or nose, skin lesions, abnormal breathing, difficulty with ambulation, decreased food or water intake, or self mutilation.
B. The frequency of observation will be increased when animals exhibit the above or other signs of moribundity. Monitoring on weekends and holidays may require involvement of the investigative staff to supplement that provided by the animal care and veterinary staff. Designated personnel, including a veterinarian, should be notified as soon as animals show signs of disease. An assessment of the animals' condition should be made as soon as possible and a plan of action established.
C. Consideration will be given to moving animals to individual cages when their condition deteriorates to the point that injury from other animals is likely.
D. Supportive care including wet feed, nutritional supplements, fluid supplementation, and warming devices.
E. Written records will be kept of monitoring.

General endpoint references:

Canadian Council on Animal Care (1998), *guidelines on: choosing an appropriate endpoint in experiments using animals for research, teaching and testing*. Ottawa, Canada.
Hendriksen and Morton, ed. (1998), *Humane Endpoints in Animal Experiments for Biomedical Research*. Proceedings of the International Conference, 22-25 November 1998, Zeist, The Netherlands. Laboratory Animals Ltd, by Royal Society of Medicine Press Limited, London, England.
Institute for Laboratory Animal Research Journal (2000), *Humane Endpoints for Animals Used in Biomedical Research and Testing*. 41: No. 2
Morton and Griffiths (1985), Veterinary Record 116:431-43.
Montgomery (1990), Cancer Bulletin 42:230-237.
Toth (1997), Contemporary Topics 36:44-48.
Stokes (1999) *Humane Endpoints in Animal Experiments for Laboratory Animals Used in Toxicity Testing* Proceedings of the 3rd World Congress on Alternatives and Animal use in the Life Sciences, 31 August - 2 September 1999, Bologana, Italy.
United Kingdom Co-ordinating Committee on Cancer Research (1997), *UKCCCR Guidelines for the Welfare of Animals in Experimental Neoplasia*, 2nd ed. London, England.

Tumor size references:

Bullard et al. (1981), J. Neuropath. Exp. Neurol. 40:410-427.
Tomayko and Reynolds (1989), Cancer Chemother. Pharmacal. 24:148-154.

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Sung et al. (1993), Cancer Research 53: 2092-2099.
Welch et al. (1994), Oncogene 9: 255-262.
Hamm (1995), Contemporary Topics 34:69-71.

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Selected Clinical Observations Used in Cancer Research and Toxicological Studies [1]

PARAMETER	WHAT TO LOOK FOR ...
General Appearance	Dehydration, decreased body weight, missing anatomy, abnormal posture, hypothermia, fractured appendage, swelling, tissue masses, prolapse, paraphimosis
Skin and Fur	Discoloration, urine stain, perianal soiling, pallor, redness, cyanosis, icterus, wound, sore, abscess, ulcer, alopecia, ruffled fur, necrosis
Eyes	Exophthalmos, microphthalmia, reddened eye, discharge, opacity, blepharospasm, proptosis
Nose, Mouth, and Head	Head tilted, nasal discharge, malocclusion
Respiration	Sneezing, dyspnea, tachypnea
Urine	Discoloration, blood in urine, polyuria, anuria
Feces	Discoloration, blood in the feces, lack of feces, softness/diarrhea
Locomotor	Hyperactivity, coma, ataxia, circling, muscle tremors, seizures, paresis, paralysis

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Adapted from Montgomery, C.A. Jr. (1990), Cancer Bulletin 42:230-237
and appeared in AWIC Newsletter, Spring 1995 6:4

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