Module 08 - Pain, Distress and Endpoints

Preface.

This module is devoted to the experience of pain and distress, and the approaches and methods we can use to evaluate that experience in animals. A subsection is devoted to the issue of setting more humane endpoints so that the pain and distress in invasive experiments is minimized. The anatomy and physiology of nociception is covered in the following module on analgesia. Nociception is the scientific term used to describe the neuro-physiology of pain perception, including the nerve pathways involved with transmitting messages to the central nervous system that are interpreted as "pain".

The objectives of this section are to:

- outline the sources of pain
- outline how we can obtain evidence of the pain experience in animals
- describe stress and distress in animals
- outline how we can obtain evidence of the distress experienced by an animal
- present some signs of pain and distress in various animal species

Pain and Distress in Animals

Introduction

Pain, stress, distress, fear and anxiety are sensory states that people are able to describe. Pain is the word we use when something hurts e.g., after an injury of some kind, an infection, a headache or sometimes an emotional event. We feel stressed when we are harried, when there seems to be too little time to do everything and so many demands are being made on us, or perhaps when you have to take a course before you can work with animals in your research program!! Usually, we are able to deal with the situations. However, when the level of stress reaches a point where we are unable to deal effectively with it, our responses may become counterproductive to our own well-being and we become distressed. Fear and anxiety are important modulators of the pain experience. Freedom from fear can reduce the pain experienced

in humans and animals, which is why tranquilizers are useful adjuncts to analgesia or anaesthesia.

Definitions and Terminology

The following definitions of distress, discomfort and pain were developed by CCAC based on FELASA (Federation of European Laboratory Animal Science Associations) definitions.

Distress: Distress is a state associated with invasive procedures conducted on an animal, or with restrictive or other conditions which significantly compromise the welfare of an animal, which may or may not be associated with pain, and where the animal must devote substantial effort or resources to the adaptive response to challenges emanating from the environmental situation.

Discomfort: Discomfort is viewed as a mild form of distress.

Pain: Pain is an unpleasant sensory and emotional experience associated with actual or potential damage or described in terms of such damage.

Suffering: The term "suffering", as in "pain and suffering" is not used in this module in relation to animal experiences, because for some animals (the lower vertebrates) that capacity may not be present in the way that humans perceive it. Instead, the word "distress" is used.

Pain and distress in a research animal may be very difficult to assess. We cannot ask the animal how it feels, and so must rely on other means of determining the level of pain being experienced by an animal. Although animals cannot communicate with us verbally and describe their sensations, they respond to pain, stress, fear and anxiety in ways similar to humans. They may show fear and anxiety when confronted with a predator, including us, and they show signs of pain when they hurt (e.g., not using a limb when it has been injured). Novel or strange situations may be stressful, as is isolation for a sociable animal. A stressful situation that is not resolved may progress to a state of distress. For example, inappropriate housing conditions that

do not permit a normal range of behaviours may result in the development of purposeless behaviours like pacing or weaving.

Even if there are no obvious signs of pain or distress, we should not assume that the animals are free of pain or distress. We may simply not be recognising the signs. We are trying to make an assessment on a totally different species when we often cannot make such as assessment on other humans.

The challenge for us is to identify the signs that suggest an animal is in pain or distress. And even if we cannot identify the signs, we should not assume that all is well. Animals, particularly species that have evolved as prey species, will successfully hide signs of pain and stress if it is not in their best interests and if they are able. For example, it is difficult for an animal in the wild to disguise the fact that it has a broken leg but it may disguise other signs of injury or disease to mislead a potential predator. In the lab, mice that have had surgery to implant ova in the fallopian tubes will behave normally if observed soon after they recover from the anaesthetic. This is seen even when a gaseous anaesthetic is used and the animals are mobile within minutes of turning off the gas. However, if the animals are observed without them knowing, they may be sitting apart from the group, licking or scratching at the wound, stretching frequently, etc. The signs may indicate that the animals are having some pain or discomfort.

Pain, stress and distress produce some similar behavioural, physiological and biochemical changes - which we know as the "fight or flight" mechanism. These changes are the result of a stimulation of the hypothalamic-pituitary-adrenal (HPA) axis, and include alterations in blood pressure, heart rate, or a change in behaviour.

There may be a wide variation in response to a painful or stressful situation in different species and between animals of the same species. Some animals will vocalize if they are hurt and the sounds will be quite different from those usually associated with the species. A guinea pig that is hurt will squeal and try to get away. It will rarely ever bite. A rat, on the other hand, will squeal and try to run away, but it will also bite. There is evidence that different strains of mice respond differently to painful stimuli. Furthermore, a strain that reacts at a low intensity for one stimulus may resist a second stimulus until it reaches a high intensity.

It is also important to emphasize the temporal nature of pain. Pain is not constant or consistent. It changes all the time both in intensity and form, so the appropriate treatment must accommodate these changes. Birds may show acute pain by vocalising and wing flapping.

Open-mouthed breathing may occur and if the pain persists, feathers become ruffled through lack of preening. A decrease in activity and protection of an injured part may also be seen. In some birds, a state of immobility and unresponsiveness may develop. Chronic pain is also seen in birds (e.g., heavy broilers with arthritis; these birds will respond to analgesics by being more mobile and limping less).

Pain in amphibians, reptiles and fish is still a poorly studied subject. However, since they have similar anatomical, physiological and neurological features to mammals and since they respond to aversive stimuli, it may be concluded that they feel pain. Some published guidelines on pain and analgesia in birds and fish are available. The effectiveness of various analgesic drugs in these animals has received limited study.

Some animals cope with stress better than others. Some coping mechanisms are internal (e.g., hormonal responses to stress), while some are external (e.g., somewhere to hide may be the coping solution when confronted by a predator, including a person). If there is nowhere to hide in a small barren cage, this coping mechanism may fail to relieve the stress and the animal will move quickly from being temporarily stressed to being distressed.

In this module, we will look for evidence of pain, stress and distress from four angles.

1. Is there any **situational** evidence that pain, stress or distress could or should exist? Has there been an injury that makes us believe that the animal must be in pain?
2. Are there any signs that the animal is **behaving** in an abnormal manner? Has it stopped grooming and looking after itself?
3. Are there **physiological** changes that suggest the animal is in pain or distress? Has the respiration rate increased?
4. Are there **biochemical** changes that indicate that the animal is stressed, distressed or in pain? Have cortisol levels increased beyond normal levels?

We should be able to gather evidence from the situation and the animal's behaviour without interfering with the animal. However, physiological and particularly biochemical evidence may require restraining the animal and taking blood or other samples. These actions may exacerbate any pain or distress the animal is experiencing and so the findings must be interpreted with care.

Although pain, stress and distress will be discussed separately, it will soon be noted that many of the signs overlap. This is to be expected since stress is a common factor in pain and distress. The object is to provide the student with a framework within which it will be possible to identify factors that could have a profound effect on experimental results. Even some of the simplest and common procedures in the animal facility may cause the animal some stress (e.g., changing cages, turning on lights).

Looking for Evidence of Pain in Animals

Situational Evidence

Our own experience with pain and distress can tell us something about an animal's experience, because there is an evolutionary continuum from animals to man, not only with respect to the physical, but also with respect to the behavioural and psychological systems. Situations that cause us pain probably cause pain in animals as well. However we must approach interpretation of animal thinking and behaviour with caution. This is sometimes called "critical anthropomorphism". Animal models have been used extensively to evaluate and test drugs with antinociceptive properties, and to determine the mechanisms whereby pain relief is obtained. This clearly shows that we believe that animals have the same, or a closely similar, neural substrate subserving pain processes, as do humans.

Pain is usually associated with physical damage to tissue. Pain is the feeling we experience when pain receptors are stimulated in the tissues. Most tissues have pain receptors and the density varies from tissue to tissue. The skin is well supplied with pain receptors while deeper organs tend to have fewer receptors. Skin incisions are painful, as we know from experience. As a result of tissue damage, chemicals are released that either stimulate pain receptors directly (e.g., histamine, bradykinin) or sensitize them to stimulation (e.g., prostaglandin). The brain is believed to be devoid of pain receptors, so injuries to the brain itself are not painful. However, other tissues within the skull do have pain receptors (e.g., arteries) and so may cause "headaches".

Traumatic damage to tissues from a surgical procedure is usually easily recognized. It is important to note that pain and stress can be significantly reduced by good surgical technique and planning so this should be addressed before rather than after the event. A major factor in perioperative pain is surgical skill. We should not substitute analgesics for incompetent surgical

technique. Trauma may occur as a result of a slip or a fall and the external evidence may not be so obvious.

Infectious diseases cause tissue damage and the resulting inflammation is part of the body's attempt to repair the damage. Conjunctivitis, cystitis and mastitis are examples of infectious diseases that are associated with pain but not all infectious diseases have a pain component. Some immune diseases may result in tissue damage (e.g., some forms of arthritis).

Compounds injected into animals may cause tissue damage and pain. Some drugs are known to cause pain when injected. For example, the pH of the solution may not be appropriate. There may be two components to the pain, one associated with the drug itself and one associated with the injection of a relatively large volume into a closed compartment for example a muscle bundle. The latter should not be a factor if the proper volumes for injection are followed. (See **www.eslav.org/efpia.htm**)

All of these examples provide us with evidence that pain could be expected. However, they do not give any idea of how much pain is occurring. That depends on a number of factors that vary the response to pain in different species, different strains, different temperaments, etc.

Behavioural Evidence

The most easily acquired evidence of pain in an experimental animal comes from noting changes in behaviour. Animals change their behaviour in response to pain, particularly pain that persists. The changes depend on the level of pain, the tolerance of the animal, the species and even strain of the animal, the situation under which the pain occurs and a variety of other factors. Short-term pain (e.g., an injection) may be very well tolerated, depending on the site of injection and the substance injected. There may be no behavioural changes as a result of a subcutaneous injection of a non-irritating material. On the other hand, a subcutaneous injection of an irritating material may result in the animal scratching at the site of injection. This repeated scratching at a specific site constitutes a change in behaviour. The same reaction may be seen in animals infected with mites that bite and irritate the skin.

Severe short-term pain may produce an aversive reaction in an animal. A normally compliant, friendly animal may attempt to bite someone causing it pain. Alternatively, the animal may attempt to flee from the cause of the pain.

Chronic or long term pain (e.g., arthritis, orthopedic procedure) is likely to produce more subtle behavioural changes. Animals and people react to pain through coping mechanisms that are both internal and external. There are descending pathways from the brain to the spinal cord that will inhibit neuronal activity and reduce the sensation of pain. In addition, compounds are released that have opioid effects (e.g., endorphins and enkephalins). These internal reactions to pain will reduce the external behavioural changes. Animals in pain will often withdraw from their social group, choosing instead to remain alone, to be less active and less responsive to external stimuli. Sometimes, persistent pain will cause an animal to traumatize further the area that is hurting. An extreme form of this is seen in animals that have undergone the severing of nerves to the foot. A neuroma frequently develops and this causes pain that seems to come from the denervated foot. The animal's response may include chewing the toes as it attempts to relieve the pain. More often, persistent pain will result in the animal scratching at the site. In our own experience, a persistent itch will cause us to scratch repeatedly at the site.

A second complicating factor is that many laboratory animals are nocturnal and so are asleep during the part of the day when we are working and the lights are on. Again it may be difficult to discern signs of pain. However, switching off the lights and using a red light to observe them can overcome this difficulty. Most of these nocturnal animals will become active within a few minutes of the lights being switched off. Animals that are inactive or those that remain separated from the group may be in distress or pain. It is possible to see abnormal postures such as hunched back or tiptoe gait that may also point to pain.

Food and water intake is often altered when an animal is in pain, regardless of the source of the pain. Because many animals used in science are housed in groups it is difficult to detect these changes. Loss of body weight can be used as an indirect measure of a decrease in food and water intake. Animals expected to develop pain can be closely monitored to allow accurate measurement of body weight.

Grooming is an important activity for animals and a failure to groom is an early sign of pain. The hair may be standing up (piloerection or staring) rather than lying down smooth. It may be dull rather than shiny and it may be matted or clumped, particularly around the face and mouth and the anal and genital openings.

Physiological Evidence

Painful experiences stimulate the hypothalamic-pituitary-adrenal (HPA) axis. Many of the physiological changes seen in pain are a reflection of the release of adrenalin and noradrenalin. Some of these changes may be observed without handling the animal. Dilation of the pupils and an increase in respiration rate may be directly observed. Measuring the body temperature, heart rate or blood pressure in small laboratory animals usually requires some interventions such as restraint or previous surgery to implant recording devices. Physiological evidence of pain is best observed in the early stages after a painful stimulus. As the pain persists, the changes tend to lessen so that the changes in heart rate, respiration rate etc. are not so dramatic and may be within the normal range.

Biochemical Evidence

Several biochemical changes accompany painful stimuli. However unless the animal has been previously catheterized so that blood can be collected during the painful stimulus, these changes are not likely to be observed. Some of the compounds or their metabolites may appear in the urine. Corticosteroids (e.g., cortisol) and catecholamines (e.g., adrenaline) will be elevated, from stimulation of the HPA axis. The metabolic rate will be increased and there will be elevation of endorphins and enkephalins. There are also changes in the immune system associated with pain (and distress), some of which can be measured. The widespread effects of corticosteroids may be seen in a range of biochemical parameters, electrolyte changes, metabolic changes, immune factors, etc. This presents the investigator with the difficulty of separating the normal from the pain-induced abnormal biochemical changes.

In addition to the four sources of evidence for pain already discussed, other evidence may be useful in special circumstances. For example if an animal in pain is treated with pain relieving drugs (analgesics) and its behaviour and physiology return to normal, we feel confident that it was in pain before.

Summary

In coming to the conclusion that an animal is in pain, we need to examine evidence from each of the areas discussed. One sign alone may be sufficient to identify an animal in pain, but more often we are trying to identify more subtle signs of pain. In these cases, several pieces of evidence will add up to a correct diagnosis.

Evidence of Stress and Distress

We will look for evidence of stress using the same four areas we looked at for pain.

1. Is there any situational evidence that stress could or should exist?
2. Are there any signs that the animal is behaving in an abnormal manner?
3. Are there physiological changes that suggest the animal is stressed?
4. Are there biochemical changes that indicate that the animal is stressed?

Pain is also a stressor, so many of the same signs will be found. However, since there are other forms of stress than just pain, we need to be able to recognize these also.

Stress and Distress: Situational Evidence

Are there any reasons to expect that the animal may be under stress? Is a social animal (and most of the animals commonly used in biomedical research are social animals that are most comfortable living in small groups) being housed alone without contact with its own kind? Often animals are housed alone as part of an experimental protocol. However, the effect of this must be recognized within the context of the experiment. It is known, for example that in mice, the growth of some tumours is increased if the mice are housed singly when compared with housing in groups.

Is the environment stimulating for the animals or does it provide little opportunity for the animals to indulge in normal behaviours? A physically impoverished environment, like a socially impoverished environment is known to be stressful to many species. This is especially so if it is combined with a restriction in activity.

Are the animals new to their surroundings or have they just been transported? Animals in these situations often show signs of stress, particularly in their behaviour. And transportation may be as little as being moved from one room to the next, even within their home cage.

Some experimental manipulations that are not associated with pain may be stressful. Even picking up an animal in a careless manner or by an unfamiliar person may trigger signs of stress.

Food and water deprivation of even short duration may be stressful to animals used to good supplies of these nutrients.

Stress and Distress: Behavioural Evidence

Again the stressed animal will show some of the behavioural changes noted in association with pain although not the pain specific signs (e.g., lameness, protecting an injured part). Stressed animals may become quiet and unresponsive or they may become hyperactive (e.g., observe the frantic activity of mice when they are placed in a nice clean cage, free from any odours of their marking). In some cases, the animals may return to apparently normal behaviour, even though the stressful situation still persists. This is described as coping and is a response to stress, i.e., the animal tries to return all systems to normal.

Stress and distress: Physiological Evidence

The physiological changes described under pain are seen in animals stressed by non-painful situations although the level of change may be less. Again these changes result from stimulation of the HPA axis with the release of corticosteroids and catecholamines. Changes in heart and respiratory rates, blood pressure and metabolic rate are all seen. Studies of the immune system have shown changes including suppression of some elements of this system. This ties in well with the well-recognized development of disease in stressed animals and people. These changes in the immune system are being investigated as a subtle indicator of a stress problem when other signs have returned to normal values.

Stress and Distress: Biochemical Evidence

As indicated above, there are raised levels of corticosteroids and catecholamines in stress. These may return to normal, even in the continued presence of the stimulus, and so their absence cannot be considered evidence of no stress. Distress may be considered to be the state of an animal that has been unable to cope with stress and where its responses not only fail to alleviate the stress but also are detrimental to the animal's wellbeing.

The eating and drinking habits of animals in distress are usually altered. Frequently they refuse to eat and drink and remain passive and unresponsive to their environment.

The physiological signs of distress are quite variable. They include the signs of stress given above but modified depending on the success of coping mechanisms. In addition, the changes in behaviour, particularly in eating and drinking will result in weight and hydration changes,

depending on the duration of the distress. There may be changes noted in some of the formed elements of the blood, particularly eosinophils, neutrophils and lymphocytes. Distressed animals may be more susceptible to infectious diseases.

As with the physiological signs, the biochemical signs may be quite variable but are similar to those seen in stress. Since the HPA axis is intimately involved, there may be changes in adrenal gland secretions and even exhaustion of adrenal function may occur.

Signs of Pain and Stress: Summary

The following table summarizes the signs of pain and stress and gives an expectation of their occurrence. Since pain and stress are seen in different intensities, the animal's response depends on the species, age, situation and a number of factors. This table is presented as a checklist of things to look for rather than a definitive or complete description. Additional information on the species specific signs of pain is available on the *CCAC web site http://www.ccac.ca/*

Sign	Pain	Stress
Protecting injured part. e.g., limb, abdomen	Commonly seen; may be reluctant to move	Seldom seen
Vocalizing	Especially if forced to move	May occur in isolated stressed animal
Respiration	Rate increased and may be shallow	Rate may be increased if animal is also fearful
Attitude	May be depressed and unresponsive to stimuli	Usually alert and responsive, sometimes depressed
Food and water intake	Usually decreased	Often decreased; some stressed animals may overeat
Urination and defecation	Reduced volume and frequency	Both may be increased with diarrhea sometimes
Appearance	Unkempt, piloerection, reduced self care	Unkempt, piloerection and reduced self care
Eyes	May be sunken, occasional	Discharge especially in rats

	discharge	and mice

It is our responsibility, when animals are used for research, teaching and testing to ensure that pain is minimized, including for those studies in which pain may accompany the research being done. We should seek evidence for pain, stress and distress in animals from several points of view. Situational and behavioural evidence may be found by observation while physiological and biochemical evidence requires the collection of information by handling or otherwise manipulating the animal. Since there is no single parameter that defines any of the states, we must synthesize our opinion based on all available evidence.

Assessment of Pain and Distress in Animals with a View to Setting More Humane Endpoints in Invasive Studies

The objectives of this section are to:

- define the term "endpoint" in relation to minimising the potential for an animal to experience pain or distress in invasive studies
- present the ethical and scientific concerns important in choosing the appropriate endpoint in a given study
- describe the observations upon which endpoints can be based
- make reference to recommended endpoints in the published literature, and provide research examples
- list some questions that can help the principal investigator and the animal care committee choose the appropriate endpoint in a given invasive experiment, and help ensure that no animals will go past the endpoint and thus experience unnecessary pain or distress

Introduction

When some level of animal pain or distress may be inherent or unavoidable in a research program in relation to a condition being studied (like cancer or infectious disease), or as an undesirable side effect of the procedures, it is our responsibility to minimize that pain or distress. Anticipating the expected pain and formulating a pain control plan are important. One way of

limiting the potential pain and distress is to decide ahead of time at what point such an experiment will stop, to avoid pain and distress that would occur if the experiment continued.

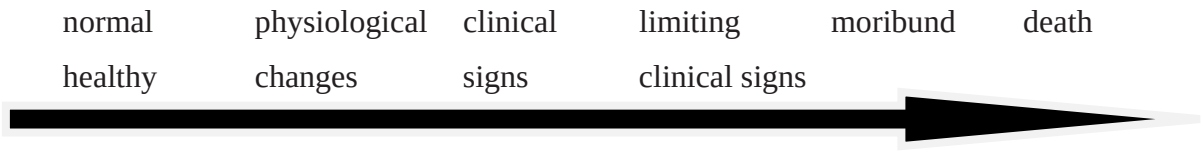
When an invasive research project is being planned, the principal investigator should consult the CCAC guidelines on: choosing an appropriate endpoint in experiments using animals for research, teaching and testing at http://www.ccac.ca/ to ensure that choosing the appropriate endpoint is done in compliance with the CCAC guidelines.

Defining "Endpoint"

For our purposes, the term "endpoint" can be defined as the point at which an experimental animal's pain and/or distress is terminated, minimized or reduced by taking actions such as humanely euthanizing the animal, terminating a painful procedure, or giving treatment to relieve pain and/or distress.

The general guideline in the CCAC endpoints guidelines CCAC guidelines on choosing an appropriate endpoint in experiments using animals for research, teaching and testing states: "In experiments involving animals, any actual or potential pain, distress, or discomfort should be minimized or alleviated by choosing the earliest endpoint that is compatible with the scientific objectives of the research. Selection of this endpoint by the investigator should involve consultation with the laboratory animal veterinarian and the animal care committee." This statement acknowledges that there are both ethical and scientific considerations that go into finding an appropriate endpoint. Arriving at that decision involves the researcher and the people at the institution given the responsibility for acting on behalf of the animals used in research teaching and testing.

The various stages of an animal's condition in an invasive experiment can be depicted on a scale from "normal" through "moribund" to death at the other end of the scale.



When a procedure affects an experimental animal, its condition starts to change from being a "normal" healthy animal. In studies that involve infection, cancer, or arthritis, for example, as the

condition progresses there may be increasing pain and distress. Eventually the condition of the animal may reach a point where it becomes obvious that unless action is taken to terminate the condition, the animal will go on to die. This point on the scale is called the "limiting clinical signs".

One example of this comes from the regulatory safety testing literature involving the rabies vaccine challenge test in mice (used to test the efficacy of a batch of rabies vaccine). The traditional endpoint for this test has been death in the control animals (and perhaps in some dilutions of vaccinated animals). Using a 4 stage clinical scoring system:

Score 1: ruffled fur, hunched back; Score 2: slow movements, circling plus >15% weight loss; Score 3: trembling, shaky, convulsions; Score 4: lameness, paralysis, permanent recumbency;

The researchers found that all mice progressing to score 2 went on to die. These observations were the most significant predictors of further deterioration in the animal's condition, and a score of 2 was the earliest point at which those signs appeared. Therefore the experimental endpoint could be set at a score of 2 rather than waiting until the mice died, without affecting the outcome of the test. When the cardinal signs of the condition are unknown, a pilot study using a few animals under close observation may provide the information to allow the earliest endpoint to be found.

Ethical and Scientific Concerns in Choosing an Appropriate Endpoint

The above example also raises the issue of the balance between scientific concerns and ethical ones in choosing an endpoint. Selection of an endpoint by the investigator is important because he/she has defined the scientific objectives, and if those are not met because an experiment was terminated too early, then the study and the animals' lives are wasted. The endpoint should not change the outcome or invalidate the results. The objective should be to have a scientifically valid experiment, while at the same time holding any pain and distress to a minimum.

Which Observations of Behaviour and Physiology are Best for Selecting he Endpoint?

There is no single answer for this question. Each research project where an endpoint is defined to minimise animal pain and distress will probably have a distinct set of observations

needed to accurately identify the animal whose condition has progressed to the endpoint. A study of the pain of castration in lambs will use different observations than a study of bacterial infection in mice. Nevertheless the approach to making (and recording) the observations will be much the same.

For most studies, there are five areas in which observations of the animal should be made:

- external physical appearance
- changes in behaviour (both when the animal is at rest and when it is stimulated)
- changes in body weight (and related changes in food and water intake)
- body temperature
- changes in clinical signs (e.g., heart rate, respiratory rate, etc.).

Of these, measuring and recording body weight and body temperature should be considered for almost every endpoints assessment.

A scale can be set up for each observation (for parametric signs) with increasing changes from normal identified, and tracked by using a checklist to record the observations. That way the changing condition of the animal can be followed from one observation time to the next. This approach also helps ensure that the observations are as objective as possible. An endpoint can then be pre-set; that point when the scoring of the animal's condition has reached the endpoint.

There are two types of observations that can be made:

1. Changes from normal (also called parametric signs) on a continuous scale from normal. These observations include: body weight; body temperature; heart rate; respiratory rate; level of activity or other behaviour. For example a scale could be set up for body weight as normal, 10% weight loss, 20% weight loss; 30% weight loss.

Some of the technologies that assist in the recording of these important observations include infrared thermometers, implantable physiological recording and telemetry microchips, computerised activity monitors, etc.

2. Presence or absence of signs (also called non-parametric signs). Signs such as ruffled coat, closed eyelids, nasal discharge, limping, hunched, recumbent, circling,

vocalisation, self-trauma, diarrhea, dyspnea, seizures could be recorded as being present or not. Viewing the recording sheet over several observations would reveal if an increasing number of signs were present.

To be effective a checklist should be specific for each experimental protocol, for each species, and strain, should list all the common and the cardinal signs in order of observation, and each sign scored as present or absent, or degree change from normal. The CCAC endpoints guidelines provide additional information on the process for developing such checklists.

A Sample Clinical Scoring Checklist

The checklist for the clinical and physiological observations made in an acute pneumonia model in cattle used to develop vaccines, included:

- a rhinitis or nasal score (0 normal to 4 very severe rhinitis),
- a respiratory distress score (0 normal to 4 very severe respiratory distress),
- a depression score (0 normal to 4 moribund),
- and a strength score (0 normal to 4 recumbent, unable to rise).

In addition temperature and body weight were recorded. When an animal reached an overall sickness score of 3 that was set as the endpoint and the animal euthanized. This choice of endpoint was based on early experience with the model.

Some Endpoints Recommendations from Published Guidelines

Body Weight Changes

The rate, duration and extent of weight loss are all important. A weight loss of 20% is considered an endpoint, in the CCAC endpoints guidelines. The UK Coordinating Committee on Cancer Research (UKCCCR) recommends that weight loss exceeding 20% should be one of the endpoints in cancer research. A number of other endpoints for animal models of cancer research are also presented in this publication. For example, the total mass of the tumour should not exceed 10% of the normal bodyweight for that animal. (CCAC guidelines on: choosing an appropriate endpoint in experiments using animals for research, teaching and testing.

Body condition scoring may be necessary to identify loss of body condition in cancer studies where the general weight loss may be cancelled out by the growth of the tumour.

Body Temperature Changes

A number of studies have shown that in mouse models of infectious disease (bacterial and viral), animals whose body temperature dropped more than 6°C went on to die, and so this would be one of the recommended endpoints in studies of this nature.

Change in Activity Level

Lethargy, depression, and sleepiness accompany many disease conditions (produced in part by the actions of cytokines released during the acute phase response). In rodents observing this decrease in activity may require observing them during the dark phase of the room's lighting system. This can be mimicked by turning the room lights off and observing the activity level under red light (the red light test).

How Often Should the Observations be Made?

The CCAC endpoints guidelines recommend a minimum of two or three observations each day during critical periods, and more frequently if necessary to ensure that no animal's condition progresses past the set endpoint. In pilot studies, continuous monitoring using video equipment can be helpful in identifying critical times.

Assisting the Principal Investigator, the Animal Care Committee and the Veterinary and Animal Care Staff in ensuring that CCAC Guidelines on Endpoints are satisfied.

The challenges for principal investigators include: setting the earliest scientific endpoint possible; defining limiting clinical signs; using best technologies for obtaining necessary observations. The challenge for animal care committees is to balance the requirements for good science, with the responsibility to minimise pain and distress. This would include pressing for earlier, data driven endpoints whenever possible. The challenges for veterinary, animal care and research staff include: ensuring careful, objective monitoring of all animals; documenting observations made; identifying animals nearing pre-determined endpoints. To help meet these challenges, asking the following questions will hopefully generate the discussion needed to meet

our collective responsibilities in ensuring that experimental animal pain and distress are minimised in invasive studies.

What are the scientific justifications for using the proposed endpoint?

Scientific justification for a proposed endpoint is related to the goals of the project. The work must have scientific merit, and the endpoint must fulfill the scientific requirements. Scientific justification should not rest wholly on comparison with published data, as this does not permit refinement of endpoints. A pilot study might be used to compare a new scientifically justifiable endpoint with data from a previous study using an older, later endpoint. There may be some studies in which going beyond normally accepted endpoints could be scientifically justified, for example, cancer treatments or treatments for other serious diseases. For these to be acceptable from a welfare point of view, the animals would need to be treated as if they were in intensive care, and provided with all possible measures to alleviate their pain and distress while allowing the study to proceed.

What is the expected time course for the animals, from the initial treatment to first signs of pain/distress to the death of the animal, based on previous information with the specific model under study?

When this knowledge is not available from previous studies, a pilot study with a few animals might provide a means of assessing the time-course of events during a study, to predict the time at which the effects on the animals are the most severe, and the times at which the animals need the most careful monitoring. This information is needed to decide when the most intensive monitoring of the animals should take place so that the endpoint is reached when the relevant personnel are present and can terminate the experiment.

When are the effects to the animal expected to be the most severe?

Knowledge of the time course of the development of a tumour, or infectious disease can also assist in determining when the animals require the most attention. Conditions that are acute, i.e., that progress to severe dysfunction or death in a short period of time, are of particular concern. Providing special care for animals whose condition is severely compromised would help ease the pain and distress they experience.

If the course of the disease and expected signs of the adverse effects are unknown, could an initial pilot study, under close observation by the investigator and/or laboratory animal veterinary staff answer these questions?

Observation by the investigator should ensure that the necessary scientific objectives are being reached, while the laboratory animal veterinary staff can provide the expertise with regard to clinical signs of pain and/or distress.

Has a checklist of observations, on which the endpoint will be based, been established?

If not, the pilot study affords the opportunity to compare observations of control animals with treated animals and to identify indicators that can be used to establish the earliest possible endpoint.

Who will monitor the animals (identify all responsible) and keep records?

All the persons involved in the care and monitoring of the animals should be identified at the outset, and must be skilled at recognizing signs of pain and distress.

Has a clear chain for reporting observations been established?

It is crucial that the individual(s) responsible for monitoring the animals have a clear reporting line so that the individual with responsibility for deciding on the termination of the experiment is informed promptly of changes in the animal that indicate the selected endpoint is imminent.

What will be the frequency of animal observations: a) during the course of the study; and b) during the critical times for the animals?

The animal care committee must be assured that the animals are going to be monitored with sufficient frequency to enable the staff responsible to identify any animals approaching the endpoint.

Do the investigator(s), animal care and technical staff have the training and expertise to monitor the animals adequately?

The animal care committee must also be assured that the individuals responsible for monitoring the animals have the training and experience to do the monitoring.

What provisions have been made to deal with any animals that show unexpectedly severe signs and symptoms?

Provision should always be made to deal with unanticipated pain and/or distress.

For toxicological studies, have existing toxicological data been evaluated?

Data mining: It may be possible to predict clinical signs from background data or databases for similar chemicals or substances. Information from human or veterinary clinical practice with similar substances may also be useful.

Summary

As noted in the CCAC endpoints guidelines, in experiments involving animals, any actual or potential pain, distress, or discomfort should be prevented, minimized or alleviated by choosing the earliest endpoint that is compatible with the scientific objectives of the research. Any distress or pain experienced by the animals in the course of biomedical research, teaching and testing, that is not necessary to achieve the scientific objectives of the research should be avoided. That is our ethical responsibility.