

Humane endpoints in cancer research

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Summary

Laboratory animals have been used extensively as experimental models to advance our understanding of the causes of cancer and develop improved treatments. Animals with experimentally induced cancer may experience distress which can be minimized by humane and experimental endpoints incorporating: (i) a knowledge of the biology of the tumours; (ii) the tissues and organ systems affected; and (iii) clearly defined experimental and humane endpoints. This presentation reviews some of the issues described in the United Kingdom Coordinating Committee for Cancer Research (UKCCCR) Guidelines for the Welfare of Animals in Experimental Neoplasia, and proposes modalities by which those involved in laboratory animal welfare can develop experimental and humane endpoints that maximize scientific benefit and minimize animal suffering. An understanding of the growth characteristics of the tumour line, the site of origin or inoculation, the tissues and body systems affected, and the consequences for the animals are essential for the development of humane endpoints in cancer studies. Components of protocols for humane endpoints will be evaluated.

Cancer is a general term encompassing a number of serious diseases characterized by disorganized cell growth and invasion and destruction of the surrounding tissues. Cancer can arise in most tissues of the body and can occur in both humans and animals. The incidence of cancer worldwide is projected to continue to increase (Boyle 1997). Where effective anti-cancer treatments do exist they can be very demanding on the patient.

Against this background, scientists engaged in experimental cancer research endeavour to: (i) discern the biological mechanisms leading to the development of cancer; (ii) detect potential carcinogens in the environment and provide protection from these agents; (iii) identify those at particular risk of developing cancer; and (iv) develop ways to cure or control clinical disease in humans and animals.

Animal models are used in cancer research to bridge the gap between understanding cancer at the molecular and cellular level, as well as how the disease develops in a complex living organism. Animals are also used

to test the safety and efficacy of anti-cancer therapies. Where these studies involve the intentional induction of cancer in experimental animals there is the potential for the animals to experience discomfort or distress. In addition, the experimental challenge and scientific techniques used to induce and monitor the experimental condition may further compromise the well-being of an experimental animal.

Laboratory animals used in experimental oncology justify special consideration from those responsible for their use and welfare. In 1988 the UKCCCR published *Guidelines for the Welfare of the Animals in Experimental Neoplasia* (UKCCCR 1988) and a revised edition in 1997. The diversity of the scientific objectives and experimental tumour models used in cancer research frustrate any attempt to produce specific guidelines. For example, the experimental objectives, potential adverse effects and humane endpoints for mice bearing solid tumours for serial transplantation will differ from those

for animals bearing similar solid tumours but being used in experiments to evaluate the efficacy of anti-cancer agents. This paper will address some of the main issues associated with developing humane endpoints for animals bearing experimental tumours.

The development of endpoints requires a careful examination of: (i) the experimental objectives and scientific endpoints; (ii) the characteristics of the tumour system and the possible adverse effect on the host; (iii) the effect of any additional experimental challenge; (iv) the onset of critical phases (e.g. tumour-associated disease, lethality or drug toxicity); (v) the animal model; and (vi) the quality of animal care. Whenever a tumour line with different characteristics is used or the experimental challenge or objectives change, then the experimental and humane endpoints may need to be amended. Experimental and humane endpoints should precede intentional moribundity or death. Tumour regression, tumour growth delay or clonogenic survival of tumour cells are preferred alternative endpoints to moribundity or death.

Experimental objectives and endpoints

Clearly defined experimental objectives help to determine the earliest experimental and humane endpoints. Biological indicators that denote experimental success or failure and which precede any significant animal suffering, should be sought. Where animals are being used to maintain transplantable, superficial, solid tumours, or to determine whether a substance or cell line is oncogenic, then the appearance of a tumour of a predetermined size may serve as both the experimental and humane endpoint. Where chemotherapy of tumours is being assayed then the experimental endpoints may not occur until some animals manifest clinical signs. Untreated tumour-bearing animals, acting as drug vehicle or positive controls, merit special consideration. Using the development of a tumour of a fixed size, or volume, as an endpoint for therapeutic assay can be problematical if the tumours develop either rapidly or extremely slowly. Where the

experimental outcome is difficult to predict or where the potential for animal suffering is high, then pilot experiments involving small numbers of animals, intensively monitored, and a staged experimental challenge can be helpful in determining both the response to experimental challenge and defining experimental and humane endpoints.

Characterizing experimental tumour models

A knowledge of the growth characteristics and biology of the tumour model and the potential effect on the animals is crucial to the establishment of humane endpoints. The tumour incidence, growth rate and the onset and nature of the adverse effects on the host will vary with: (i) method used to induce the tumours; (ii) the biology of the tumour; and (iii) the body system involved. Established methods of inducing experimental cancer in animals include exposure to carcinogenic substances, local or whole body irradiation, inoculation with viruses or malignant cells, genetic mutation and, more recently, genetic modification.

Tumours may be induced or spontaneous; solid and localized or solid tumours that disseminate (metastasize) to produce secondary disease in other tissues; liquid cancers of the blood cells, bone marrow or lymphatic system; or ascitic tumours that grow as individual cells and yield ascitic fluid. Tumour cell lines are frequently genetically unstable and subject to continual selection at a cellular level in the host, as well as through laboratory processing. Serial transplantation or transfer into a host of a different genotype may transform the characteristics of a tumour line. Typical changes in tumour biology include a reduced latent period or tumour take, and an increased malignancy or ability to metastasize (Visoneau *et al.* 1998). Detailed information on the biology of any new tumour line should precede its use in animals. Scientific protocols for the propagation of tumours *in vivo* should be followed scrupulously. Tumour cell lines should also be screened for contamination with animal pathogens prior to inoculation into animals.

Monitoring tumour development

Unexpected or uncontrolled tumour development can result in increased animal suffering. Care must be taken to establish appropriate management systems to monitor animals for the onset of tumour-associated disease. Every tumour-bearing animal should also be assessed for paraneoplastic conditions, such as cachexia. Where solid tumours grow in superficial tissues (such as mammary tumours), tumour development and adverse effects can often be easily observed. The development of ascitic tumours, characterized initially by abdominal distension, can also be easily monitored. The development of solid tumours growing internally or experimental cancers of the blood, bone marrow or lymphatic system are more difficult to monitor, and clinical signs are frequently used to confirm the development of these tumours. For some murine leukaemias, tumour-associated disease can be linked to an increase in the level of circulating cancer cells in the blood, which can be used to predict an impending deterioration in an animal's condition prior to the onset of clinical signs. However, where immuno-deficient mice are inoculated with human cancers the number of circulating cancer cells is less predictive of either the onset or the severity of clinical signs. In these cases, progress of the disease may be slower and the number of circulating human leukaemia cells may not be indicative of a poor prognosis or the onset of severe clinical signs. In the absence of validated laboratory based assays, animals should be observed for early clinical signs of cancer of the blood or lymphatic system, such as anaemia, enlargement of the spleen and lymph nodes, and before the onset of limiting clinical signs, such as consistent weight loss, anaemia, apathy or compromised respiration.

Establishing a profile for solid tumours developing internally may involve serial termination of animals, investigative surgery, medical diagnostic imaging techniques, and palpating for organ enlargement or tumour growth. All tumour-bearing animals should be autopsied to ascertain the incidence and spread of tumours or secondary deposits.

Laboratory assays for human DNA or tumour markers may be useful in detecting disseminated disease in the tissues of animals inoculated with human tumour cell lines. Histopathology can detect occult tumour cells. Laboratory assays of body fluids and tissues can be used to detect preneoplastic stages in specific organs.

Animals that are in remission and retained after anti-cancer therapy should be monitored carefully for tumour regrowth or the development of metastatic disease in other sites.

Specific welfare concerns associated with tumour development

Tissue distension

Tumour development in sites where tissue distension may result in pain or disability should be controlled. Unrestricted growth of tumours in sites such as, the muscles of the hind limbs or the cranium, can be expected to be potentially distressing. Muscular distension caused by tumour growth in the hind limbs is easily monitored either by caliper measurement or by use of templates perforated with holes of increasing diameter. Medical imaging techniques, neurological disturbance or serial killing can be utilized to profile the development of brain tumours. Consistent loss of body weight has been reported as indicating the onset of irreversible decline in rats with experimental brain tumours (Redgate *et al.* 1991).

Paraneoplastic syndromes

Tumour development may be associated with significant disturbances of the host's normal physiological or metabolic processes. Cachexia is one of the most obvious conditions and is characterized by an energy imbalance leading to consistent weight loss, and wasting of the muscles. Although cachexia is usually associated with the use of specific tumour lines, all tumour-bearing animals should be monitored for loss of condition. The intentional induction of a significant and sustained weight loss may be necessary for some experimental regimens. The aim here should be to initiate the

experimental phase as soon as possible after the onset of cachexic symptoms and before the appearance of limiting clinical signs. Humane endpoints for cachexia studies should limit the loss of weight (maximum of 30% below initial body weight) and muscle wasting, as indicated by body scoring.

Ulceration

Ulceration of the overlying tissues may occur whenever tumours are implanted subcutaneously or into the dermis of the host. Ulceration may be due to: (i) large tumours breaking through the overlying fascia; (ii) situations where large tumours develop on the ventral surface and are subject to constant abrasion; and (iii) where tumour cells are injected directly into the dermis.

Some tumour cell lines are predisposed to ulcerate, which may occur when the tumours are relatively small. Where ulceration is an inherent characteristic of the tumour line the aim should be to complete the experiment in the latent period. Once a tumour has ulcerated, its growth pattern will alter and this may be sufficient grounds for terminating the experiment. Ulcerated tissue can lead to a continual loss of body fluid, tissue necrosis and infection. Where it is necessary to keep an animal with an ulcerated tumour, steps must be taken to assess both the ulcerated tissues and the animal's general condition for any signs of deterioration.

Lethality

Some tumour cell lines can result in early lethality and measures should be implemented to establish humane endpoints prior to animals becoming moribund or dying. The L1210 mouse leukaemia is lethal around 8 days post inoculation. The terminal period is associated with abdominal ascites, dyspnoea and piloerection, and animals should be inspected several times each day and continually assessed for early termination. Where experiments involve the use of lethal cell lines then the experimental phase should be completed before the onset of tumour induced morbidity.

Large tumours

Rodents can frequently sustain large superficial tumours, up to 10% of their body weight, without any apparent adverse effects or restriction on their normal behaviour. However, it is usually desirable to place limits, consistent with the objectives of the experiment, on the tumour burden. Limiting maximum tumour development to a specific size or volume is a more immediate method than limiting the size of the tumour to a percentage of an animal's body weight. Where tumour growth restricts an animal's ability to move normally or to eat or drink then early termination is advisable. Uncontrolled development of tumours inoculated in internal organs is likely to cause distress and measures must be taken to establish the growth characteristics of the tumours and to limit development.

Preparatory procedures and experimental challenge

To ensure the engraftment of specific tumour lines it may be necessary to modify the host's normal physiological state. Low level, whole body irradiation or immunosuppressive drugs are frequently used to further suppress the immune system of severe combined immune deficient (SCID) and athymic nude mice prior to inoculation with human tumour xenografts. Surgery may be used to remove endocrine glands to assist in the establishment of hormone sensitive tumours or to implant tumour cells into the body cavities.

Experimental challenge may include novel or established anti-cancer treatments and various scientific techniques may be needed to administer the experimental challenge or to monitor an animal's response during the experiment. These additional scientific procedures may involve a greater challenge than that of the developing tumour. Humane endpoints should reflect the cumulative effect of all the experimental challenges on the well-being of an animal.

Animal models

The laboratory mouse is the most widely used species in cancer research and genetic

mutations involving the immune system, such as SCID and nude mice, make them particularly susceptible to natural infections. A number of mouse strains, notably AKR and SCID mice, have a high incidence of spontaneous tumours. Development of spontaneous tumours may cause additional suffering and result in early mortality. Genetic engineering has enabled many new strains of mice with a predisposition to cancer to be generated. The outcome of these genetic manipulations is not always predictable and tumours may occur in tissues other than those targeted. Transferring the genetic modification from one mouse strain to another can alter both the incidence and site of development of tumours.

Animal care

A high standard of animal care and specially trained personnel are essential to provide for the welfare of animals used in cancer research. Animal care staff must be familiar with the nature of the tumour system, the experimental objectives and any possible effects associated with tumour development and the appropriate endpoints for the animals in their care. Research staff must be sensitive and responsive to any concerns over the welfare of animals. Critical phases must be highlighted and the animals monitored appropriately as described above. An efficient system of assessing the quality of animal care and communicating concerns about the welfare of the animals should be established.

Distress scoring and humane endpoints

A number of systems have described specific and general clinical signs of pain and distress in laboratory animals (Morton & Griffiths 1985, Universities Federation for Animal Welfare 1989, Montgomery 1990, Wallace *et al.* 1990). Linking a numerical scale to each adverse sign enables the increasing severity and cumulative impact on the animal to be scored (Morton & Griffiths 1985). Limiting clinical signs for tumour-bearing animals are described in the UKCCCR Guidelines (1997). Integrating these distress scoring systems

into cancer studies has led to a marked refinement of humane endpoints. While these systems can reliably identify animals that are unwell, they do not automatically provide a dependable prognosis of survival or time of death. Not all adverse clinical signs are equally indicative of the seriousness of an animal's condition. Continuing body weight loss, progressive dehydration, anorexia, hypothermia and apathy, if present together, indicate that an animal is in a life threatening situation. Loss of body weight, piloerection or hunched posture indicate that an animal is unwell but do not verify the seriousness of the underlying condition. In cancer studies, emaciation may be a more reliable indicator of a serious condition than loss of body weight alone. Some scientists have expressed concern that terminating an experiment early on the basis of a series of clinical observations could lead to a loss of essential data and a waste of animals. Further progress in refining and defining experimental and humane endpoints require that distress scoring systems are subjected to rigorous validation. The association between the observed changes in the animal's condition, the underlying causes and the potential outcome must be strongly correlated.

Humans have been more closely monitored in the final stages of serious illness and death than any other species. APACHE II—a severity of disease classification system, (Knaus *et al.* 1985) uses basic physiological principles to categorize seriously ill human patients. Laboratory assays utilizing micro-quantities of body fluids or tissues and telemetry now make it possible to monitor metabolic or physiological changes in small animals. These systems could be refined and adapted to develop humane endpoints for animals under a variety of experimental conditions.

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