
Overview

Influence of Pain and Analgesia on Cancer Research Studies

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Mice and rats are valuable and commonly used as models for the study of cancer. The models and methods of experimentation have the potential to cause pain to some degree, and all charged with ensuring animal welfare must determine how to manage it. A commonly posed question, especially from investigators and IACUC, is whether the provision of analgesic agents will render the model invalid. Left untreated, pain is a stressor and has negative consequences, most notably immune system perturbations. In addition, analgesic agents in the opioid and NSAID drug classes exhibit immunomodulatory activity and influence processes such as cell proliferation, apoptosis, and angiogenesis that are important in cancer formation. Therefore, both pain and the agents used to alleviate it have the potential to act as confounding factors in a study. This review article presents data from both human medicine and work with animal models in an attempt to help inform discussions about the withholding of analgesic agents from animals used in cancer studies.

Abbreviations: COX, cyclooxygenase; NK, natural killer; VEGF, vascular endothelial growth factor

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Cancer is a simple term used to describe a complex disease process defined by unregulated cell growth, resulting in 200 different types identified by tissue of origin and location and further classified in the individual patient by stage and grade.⁷⁷ Rodent species, most notably mice, play a vital role in studies of cancer biology and therapeutic strategies.³⁸ Commonly used protocols involve the orthotopic or heterotopic transplant of xenografts or allografts into recipient mice that, to avoid transplant rejection, are either immunocompromised strains or syngeneic to the tissue donor. Rats are used to some extent, but increased costs of maintenance and limitations in genotype availability restrict their use.⁷⁸ A search of the National Center for Biotechnology Information database using terms including 'rats' or 'mice' and 'cancer' returned 139,850 results, showing the predominance of these models. The precise objectives of such studies can vary widely, but all share the common ultimate goal, which is to contribute to the body of scientific knowledge that will help identify effective treatment regimens for cancer and associated comorbidities. Reaching this goal requires that studies are translational in nature, and the models are most valid when they embody the complexities faced by physician oncologists treating human patients in the clinical setting.

Research protocols are necessarily diverse in their aims, methods, and research endpoints. For example, xenograft models, where human tumors are transplanted into recipient mice, are among the most useful and commonly used. Several carcinogen-induced and genetic-based approaches are in existence as well.¹⁰ In the case of xenograft models, tumor transplants can be established and well-characterized cells or tissues or novel clinical specimens obtained directly from human cancer

patients, also referred to as 'patient-derived xenografts' (PDX). The method of implantation into the recipient may be noninvasive—for example, by means of intravenous or subcutaneous injection, requiring minimal time and animal restraint—or much more invasive and involving major survival surgery under general anesthesia. In any model, the behavior of the cancerous cells ranges from highly aggressive and metastatic to slow-growing and localized. The result is an array of effects on the overall health and wellbeing of animals on study, ranging from nearly inconsequential, with small, local tumor growth studied at early time points, to extreme physical debilitation in studies of aggressive and metastasizing cancers that are allowed to advance. These end points are sometimes fairly predictable, and at other times relatively unknown.

Research groups are required to consider animal welfare in the course of preparing an animal use protocol, and the goal of optimizing animal welfare is often discussed during subsequent protocol review by veterinarians and IACUC (or equivalent body). Recommendations to promote welfare must occasionally be made in the face of incomplete knowledge about the behavior of the model under study. To meet this challenge, IACUC often develop guidelines and policies for humane end points to provide criteria helpful in determining when intervention is warranted. The precise intervention for a particular study is further defined and often consists of provision of palliative care and analgesic agents until a point when euthanasia takes place. The decision to treat an animal in the interest of promoting welfare seems simple enough, but because any scientific endeavor mandates the definition and control of confounding variables, the potential effect of any pharmacologic agent administration on model validity must be considered. This concern is raised often when reviewing such studies. Occasionally lost in the discussion, but important nonetheless, is an acknowledgment of the impact of untreated pain. Therein lays the challenge to

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researchers, laboratory animal professionals, and IACUC members, all of whom are obligated to ensure that animal health and welfare is addressed appropriately while at the same time striving to ensure data validity. In the end, a care and use protocol must be put into place prior to study initiation, and protocol reviewers decide whether proposed pain management is sufficient or if foregoing treatment is justified. This broad question of whether to treat has been thoroughly addressed in reference 65, and the authors provide a decision tree that might be helpful in making these determinations.

The current review article explores the use of rodents as models of cancer and the ramifications that unrelieved pain might have regarding the study outcome and, conversely, the possible effects of providing analgesia. The intent here is not to make an argument for or against any particular scheme for managing these models but instead to highlight the existing peer-reviewed literature that might inform decisions made in caring for the animals. Included are data from the human literature where they are relevant to animal models and help to provide insight into the animal experience. Otherwise, the focus will be on animal studies of tumor growth and metastasis where analgesic agents and pain were examined as variables. It is important to note that many of the studies cited are concerned with pharmacologic agents that are not routinely used in laboratory animal medicine for pain management, but similarities in mechanisms of action within a class of drug make this information relevant.

Pain, Biologic Responses, and Cancer

The degree of pain resulting from cancer induction in animals is likely quite variable and certainly challenging to quantify. The human experience sheds some light on the matter, and reports by physician oncologist colleagues can ultimately serve as a solid framework for discussions about what animals in our care undergo and thus their management. Significant psychologic and emotional stressors in human patients can also influence clinical outcomes and represent complexities that are nearly impossible to recapitulate in animal models.⁴⁴ But, in pursuit of answering questions pertaining to physical pain, humans—in something of a reversal of roles—can serve as models for what animals might experience. This role becomes especially relevant given that many studies using rodent models are translational in their aims. In addition, several regulatory principles urge researchers to consider the human condition and apply it to animal models, making human clinical data useful. Most relevant is *US Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training*, specifically principle IV, which states “Unless the contrary is established, investigators should consider that procedures that cause pain or distress in human beings may cause pain or distress in other animals.”³⁵ Stated another way, if a procedure is painful to a person, it’s likely painful to an animal.

Physician oncologists typically acknowledge that pain arises from 2 primary sources: 1) the cancerous process itself and associated comorbidities, such as paraneoplastic syndromes, and 2) the sequelae of necessary therapeutic regimens including chemotherapy, radiation therapy, and surgical resection of primary tumors.⁷⁶ Ancillary symptoms, such as fatigue, contribute as well but are challenging to relate to animal models. Regardless of origin, pain is considered to be one of the most distressing and disabling symptoms in human cancer patients.⁷⁶ There seems to be no consensus on prevalence, which is unsurprising given that studies address a variety of cancer types, therapies, and stages of progression. Overall, approximately 64% of cancer patients with advanced disease reportedly experience

pain, with estimates ranging as wide as 14% to 100% depending largely on the type of cancer under consideration.^{31,81} Comparable data are unavailable in the veterinary medical literature, although clinicians broadly agree that pain is indeed a problem and likely undertreated in companion animal cancer patients.⁴⁸

Pain can be classified in any number of ways, but a simple scheme sufficient for the current discussion identifies 3 categories: acute, inflammatory, and neuropathic.²³ An alternative approach characterizes pain by location—visceral, somatic, or neuropathic.⁶⁶ With regard to cancer, the classification becomes somewhat irrelevant, because as the disease progresses, pain is mixed in its origins, reflecting the complexities of the disease. Inflammatory mediators are released from tissue in reaction to a growing tumor and from the cancerous cells themselves, and neuropathic pain results from local invasion and compression of adjacent nerves.⁵² In human and companion animal medicine, cancer pain becomes chronic in nature and variable in intensity, dependent on the type, location, and stage.⁸⁰

Whatever the origin, type, or duration, pain is at its core a physical stressor. Pain can serve a useful purpose, like causing avoidance of injurious stimuli, but left unchecked, it is certainly undesirable, leading to distress and significant negative consequences that threaten survival. The reaction to a stressor—that is, the stress response—is highly conserved across thousands of species and is generic in that any type of stressor effectively leads to the similar, myriad changes that were first described by Dr Hans Selye and have since become well characterized.^{14,79} The most noteworthy reactions involve the activation of the sympathetic nervous system, leading to catecholamine release and subsequent elevations in heart rate and blood pressure; observable behavioral changes, for example, the classic ‘fight or flight’ response; endocrine responses mediated primarily through the HPA axis and resulting in corticosteroid release; and alterations in the population of circulating immune cells, referred to as a ‘stress leukogram’ in the clinical pathology vernacular.^{5,14} This last perturbation involving the immune system is the mechanism through which pain most likely influences the biology and behavior of cancer.

A thorough discussion about the role the immune system plays in cancer prevention is beyond the scope of this work and interested readers are directed to reference 19 for an excellent review of the subject. Briefly, 2 cell types of the innate immune system, natural killer (NK) cells and T lymphocytes, are critically important in surveying the body for transformed cells, that is, cells that have mutated and express nonself antigens.^{18,30} When these cells are identified, the full complement of immune system machinery is activated to destroy them in a way similar to how pathogenic organisms are eliminated. In some cases, however, persistent mutation allows the cell population to evade detection and continue proliferating, eventually causing clinical disease. Given the immune system’s role as the linchpin in the body’s natural ability to identify cancerous cells and prevent disease, it stands to reason that a factor such as pain that alters immune function, will affect tumor biology and behavior. It is rational to conclude that the converse is also true, which is to say that reducing or eliminating pain allows a return to immunologic homeostasis, thus reducing the likelihood of cancer development.

Precisely how these generalities apply to animal models of cancer is difficult to determine. We can reasonably hypothesize that some level of discomfort is inherent to *in vivo* cancer models and that pain, when present, becomes a variable in a study. For scientific reasons, relieving pain is the logical way to minimize its effect, but then the effect of the analgesic agent itself

must be questioned. The following discussion of findings from studies addresses these issues.

Pain, Analgesics, and Cancer Biology: Clinical Evidence in Human Patients

Human oncology teams make every attempt to optimize treatment regimens, striving to avoid steps that might exacerbate the disease and attempting to “do no harm,” in keeping with the Hippocratic oath. Patients undergo procedures and treatment regimens that are necessary for achieving a cure but that have the potential to cause considerable pain and discomfort. This treatment-associated pain is in addition to that arising from disease progression itself. The use of analgesic agents, then, comprises a significant aspect of any treatment plan. Opioids, especially morphine, serve as the cornerstone in acute and chronic pain management,⁵⁴ although several adjunctive treatment modalities, such as acupuncture, are used also.⁷⁶ This widespread use of various agents has led to several retrospective studies in the past 10 y that have questioned the role that analgesia and anesthesia regimens might play in exacerbating disease.^{4,8,12} Most of these studies involved patients who have undergone surgical procedures, which serve as a confounding variable given that surgery itself represents a significant insult to the body. This physical stressor in turn, leads to profound physiologic perturbations that in and of themselves have the potential to raise the risk for accelerated tumor growth and metastasis.^{29,57,70} It becomes difficult to separate the influence of acute surgical stress and pain from that of analgesia regimens on clinical outcome. At present, no long-term studies examining effects of chronic analgesic administration are available, and published reports are retrospective in design and of limited value.

Opioid effect. Opioids have historically been used more commonly than NSAID for the control of acute surgical pain and are mainstays in the management of chronic pain arising from disease progression. As a drug class, opioids comprise a diverse array of compounds that differ from each other largely in their affinities for the 4 opioid receptors subtypes— μ , κ , δ , and opioid receptor-like 1—which are present in distinct anatomic locations (for example, spine and brain) in different concentrations. Morphine is often considered to be a prototypical opioid and binds μ receptors most readily, whereas buprenorphine, for example, binds to μ , κ , and opioid receptor-like 1.^{2,50} The analgesic benefits of opioids are undisputed, and their extensive use in cancer pain management is warranted.

A substantial body of evidence suggests that opioids promote tumor growth and metastasis through a variety of mechanisms. The major shortcomings in these retrospective studies are the various administration protocols and a rather heterogeneous patient population, thus making it challenging to establish a clear cause-and-effect relationship.^{1,4,12,22,25,86} However, opioids have been shown to modulate immune system function, apoptosis, tumor cell invasion, and angiogenesis.^{1,3,40} Given that these systems and processes play significant roles in cancer formation and progression, expecting these drugs to directly influence the course of disease in some way is entirely reasonable. Some cancers, in fact, express μ opioid receptors, and studies of prostate and lung cancer have found that increased μ receptor expression is associated with more aggressive tumor behavior.^{75,86} It is speculated but unclear that the administration of a drug with high affinity for μ receptors, like morphine, would worsen the outcome for patients.

Controlled clinical trials examining opioid effects on cancer progression are a rarity. Fortunately, initiatives to design and

execute precisely these sorts of studies, which likely will generate data infinitely beneficial to oncologists, are in place.^{7,12,74} However, only a few reports on prospective studies are presently available. Patients undergoing surgery for colon cancer experienced improved long-term survival when they received epidural anesthesia, compared with those who received intravenous opioids for pain management. The localized compared with systemic administration presumably was responsible for the differing results, however, the study authors noted that the benefit from local administration occurred only in patients without evidence of metastatic disease at the time of the procedure.¹¹ Another study assayed vascular endothelial growth factor C (VEGFC), which is instrumental in angiogenesis in solid tumors and possibly in metastasis, in 2 groups of breast cancer patients undergoing surgical intervention.⁴⁹ One group received general anesthesia alone, and the other was administered general anesthesia with supplemental paravertebral anesthesia. The paravertebral group showed better pain control with reduced need for morphine analgesia as well as significantly lowered VEGFC levels, which would be predicted to lead to a better outcome.⁴⁹ In contrast to studies suggesting positive effects of ancillary anesthesia methods, another comparing patients undergoing abdominal surgery for a variety of cancers, mostly colon, showed no improvement in survival when epidural anesthesia was administered in addition to general anesthesia.⁵⁶

NSAID effect. NSAID may occasionally be included in a comprehensive postoperative pain management regimen in cancer patients but are used infrequently for chronic pain management. This drug class exerts its action primarily by inhibiting the activity of cyclooxygenase (COX) enzyme isoforms 1 and 2. Particular agents may exhibit a predilection for acting preferentially on either form of the enzyme or equally on both. The COX1 enzyme is constitutively expressed in many tissues and plays maintenance roles in many physiologic processes, whereas COX2 is expressed transiently in the presence of various cytokines and growth factors and is important in the inflammatory process.⁸² In both animal models and humans, various cancer types cause an upregulation in COX enzyme production and consequently may promote disease progression and hinder the detection of abnormal cells by the immune system.^{8,19,20,46,84}

Relatively few studies of NSAID use in human cancer patients have been reported. The data that do exist predominantly show that NSAID administration leads to improved clinical outcomes for patients and increased rates of survival, thus suggesting some level of anticancer action. Ketorolac is commonly used to manage acute surgical pain and has received the most attention in multiple retrospective studies.^{15,24-26,67} Several groups report that a single dose of ketorolac at the time of surgery to remove primary breast tumors is associated with increases in disease-free and overall survival times^{24,67} as well as reduced recurrence at distant sites.¹⁵ In contrast, another study²⁵ reported no effect from ketorolac administration to patients with prostate cancer. The authors postulated that the drug acted directly on cells rather than indirectly through the immune system; therefore cancers of different origins (for example, breast compared with prostate) would likely express different antigens and therefore might not be equally responsive.²⁵

Buttressing data showing the positive effect of NSAID used as analgesics postoperatively are several interesting reports regarding the benefits of aspirin when included as part of a treatment regimen or when used as a chemopreventative agent.^{16,36,72} Aspirin is not considered effective in cancer pain management, and the interest has been in low-dose, chronic administration. Several excellent reviews^{9,42,71} are available regarding the use of

aspirin as a preventative for gastrointestinal and colorectal cancers and for its effects on reducing metastasis of several cancer types. Although the precise mechanism for the observed effects can only be speculated, the benefit is likely the result of the drug acting directly on cancer cells, given the presumed absence of any analgesic effect and immune system modulation.

Pain, Analgesics, and Cancer Biology in Animal Models

Similar to the situation with humans, any pain or discomfort in a rodent cancer model could arise from 2 principle sources: 1) surgery—typically associated with the implantation of cells or tissue samples instead of therapy—and 2) local tumor growth, metastasis, and comorbidity, with acute pain arising from implantation and chronic pain accompanying local tumor growth and metastasis. Tumor inoculation can often be accomplished by using fairly noninvasive methods that require only short-term physical or chemical restraint (for example, when subcutaneous injection is used). Conversely, some orthotopic models of brain, bone, pancreatic, and liver cancer, for example, may involve a surgical procedure for implantation. In these models, management of acute pain in the postsurgical period is often practiced, with analgesic agents administered for 24 to 48 h after the procedure but not necessarily continuing for subsequent days and weeks throughout the course of disease development. One exception may be when animals approach a severe humane endpoint. Most studies of chronic drug administration are concerned with the agent's effect on cancer biology, and although several use regimens that ostensibly provide analgesia, pain measurement and management is rarely the study focus. Therefore studies focusing on cancer biology should be differentiated from those addressing solely mechanisms of cancer-induced pain, which are fewer in number.⁵⁸

Pain and opioid effect. Reports on controlled studies of pain and opioid use for their separate effects on cancer behavior in animal models are relatively sparse in the published literature. Where they do exist, the focus is often on acute surgical pain and treatment with an opioid; therefore both the indirect effect of pain and direct effect of opioids themselves are considered together in this section. The paucity of reports on opioid use to manage chronic cancer pain reflects that pain from cancer development and progression (when it exists) is probably insidious in onset and of low to moderate intensity for the duration of most animal studies. Quantifying pain in rodents is known to be challenging, but methods for measuring chronic pain are particularly unreliable, and it is unclear what assays might be most useful.^{41,55} The existing studies of the direct effects of opioids on tumor behavior use a variety of models. This situation presents challenges to making generalizations about their action, but it appears that opioids hinder cancer growth indirectly by controlling pain, but their direct effects allow tumor growth and metastasis. Although agents seldom used in laboratory animal rodents, such as morphine and fentanyl, receive the most attention, the shared group of receptors through which all opioids exert their effects support extrapolation of these findings to other research and clinical scenarios.

One method for measuring the pain effect on a cancer model is to subject animals to acutely painful stimuli, such as a surgical procedure coincidentally with disease induction, and several studies^{59,60,61} have used this approach both in rats and mice. This situation might loosely recapitulate conditions in which humans undergo therapeutic surgery, with a difference being that in of the human cases, the disease has been present prior to

diagnosis and the initiation of treatment. Several studies⁶¹ have used a Fischer 344 rat model of mammary adenocarcinoma involving intravenously administered MADB106 cells. Morphine administered at analgesic doses, as determined by a hot-plate assay and behavioral observation, concurrent with laparotomy surgery resulted in lung retention of tumor cells comparable to that in nonsurgery control animals and significantly lower than surgery controls at the 14-h time point.⁶¹ Cohorts receiving analgesic doses of morphine during the immediate perioperative period showed decreased pulmonary metastasis at 21 d after inoculation. NK cell numbers were higher in treated groups at 24 h after surgery, but those differences disappeared after several days.⁵⁹ In another study of nearly identical design,⁶⁰ the study was extended to include fentanyl and bupivacaine combined with morphine given intrathecally, with a similar reduction in lung retention of tumor cells when either regimen was used. NK activity at 5 h after surgery was significantly reduced in animals undergoing surgery compared with controls.⁶⁰ All outcomes strongly suggest that the analgesic agents used exerted their effect through the relief of surgical pain and not necessarily through a direct effect on other targets.

All of these findings are in general agreement with another investigation using the same Fischer 344 model of mammary adenocarcinoma, with buprenorphine as the opioid of interest.²⁷ In that study, the agent was administered immediately and at 5 h after laparotomy at doses thought to provide analgesia, according to previous analgesiometric assays. MDB106 cells were injected at the time of the second dose, and animals were euthanized 21 d later to measure lung metastasis. Buprenorphine only slightly increased metastasis to the lungs of animals in the absence of surgery but significantly reduced lesions in animals undergoing laparotomy. In addition, NK cell activity was slightly inhibited by buprenorphine in nonsurgery controls but was increased compared to those in the surgery group receiving saline at 6 h. Morphine and fentanyl were studied also, and each increased lung metastasis more in the nonsurgery group than either saline or buprenorphine, and both morphine and fentanyl were comparable to saline in the surgery group.²⁷ Fentanyl and morphine significantly reduced NK cell activity in the nonsurgery animals, with minimal effect in the surgery group. Using the same model in another study²⁸ with morphine and the addition of tramadol, which has moderate μ receptor binding activity, similar outcomes were reported, with each agent reducing pulmonary metastasis when administered 3 times perioperatively.²⁸ Each reduced lung metastasis slightly in nonoperated rats. Morphine reduced NK cell activity significantly in both operated and nonoperated animals, whereas tramadol increased activity in nonoperated animals and achieved levels close to sham controls when surgery was performed.²⁸

A similarly designed set of experiments with BALB/c mice undergoing percutaneous inoculation of 4T1 mammary tumor cells along with laparotomy showed that surgery led to increased tumor growth and metastasis.⁴⁷ Pre- and postoperative doses of buprenorphine administered through 24 h were most effective at reducing lung metastasis measured at 22 d. A single dose of buprenorphine had similar but less dramatic effects. Although it is reasonable to conclude that pain relief played a role in slowing tumor growth and spread, results from nociceptive and behavior testing unexpectedly indicated that animals experienced minimal pain secondary to the surgical procedure or even tumor growth, which exemplifies the complexities of these models and challenges in measuring pain in rodents.⁴⁷ Although the control of pain might have been a factor, it is also

possible that buprenorphine in and of itself increased the ability to metastasize, dependent on serial dosing.

Another approach that is reasonably reflective of chronic conditions experienced by humans is to assess spontaneous pain caused by local tumor growth and the formation of metastatic nodules over time. These models do not involve the addition of a painful surgical procedure; therefore acute pain beyond the induction of tumor growth is ostensibly minimal or absent. C57BL/6 mice injected with syngeneic B16–BL6 melanoma cells into a hindpaw experienced reduced primary tumor growth and pulmonary metastasis when receiving serial doses of morphine.⁷³ Analgesimetry suggested that animals experienced chronic pain due to cancer progression by approximately 12 d after inoculation and confirmed the effectiveness of the morphine regimen. To further support the idea that pain relief was primarily responsible for the outcome, sciatic neurectomy on the inoculated limb prior to morphine treatment yielded a similar result.⁷³ Another study showed C3H mice receiving an NCTC 2472 osteosarcoma cell line injection into the distal femur to be painful by day 7 as determined by spontaneous limb rotarod use.²¹ Morphine or fentanyl was given subcutaneously from days 1 through 14 or 7 through 17, respectively. Both regimens were effective in pain management and reduced bone lesions measured by using CT at day 18, with fentanyl exerting a greater effect. C3H/HeJ mice inoculated with the same NCTC 2472 cell line treated continuously with morphine through osmotic minipumps showed a dose-dependent increase in bone loss at day 12 compared with controls, when measured radiographically.³⁹ There was no direct effect on tumor cells when assayed *in vitro*, but osteoclast activity was enhanced in the presence of morphine, possibly leading to increased bone lysis. In addition, chronic administration of morphine appeared to result in hyperalgesia; therefore, pain stress might have also played a role.

Reports of studies on opioids in tumor models in which pain was either not intentionally induced or was absent are few. With pain absent as a variable, the direct effect of opioids on tumor growth *in vivo* might be discernible. However, if the presumption is that pain will occur in most models, then these scenarios will be rare. Nevertheless, single administration of buprenorphine, when given concurrently with intravenous inoculation of B16 melanoma cells to C57BL/C mice, had no effect on tumor seeding in the lungs when compared with saline controls assayed at 17 d.⁴⁷ This situation is in contrast to a similar study of MCF7 breast cancer cells implanted subcutaneously in nude mice, in which morphine accelerated tumor growth and increased vascular density at 28 d of growth.³² Naloxone effectively abolished this effect. Of these 2 studies, the former assayed for pain and found no evidence for its presence, whereas the latter did not attempt to measure it, although subcutaneous tumors arguably cause minimal pain at early time points.

NSAID effect. Studies examining the effect of NSAID administration on mouse models of cancer are rather numerous. The majority shows a significant impact on tumor growth and metastasis and most explore the underlying mechanism behind this effect—information that ultimately is valuable to oncologists. With the focus on translatability to human clinical conditions and improving therapeutic regimens, and given that NSAID are seldom used to control significant pain in human cancer patients, most investigations address direct mechanisms of action on tumor behavior and therefore use agents not typically administered to laboratory animals for pain management. Although this situation represents a potential knowledge gap for the laboratory animal science community, the common core

mechanism of action for this class of drugs, allows extrapolation to those that are often used, making the information useful in discussions of influence on cancer models.

Only one published report examining meloxicam for its effect in a cancer model *in vivo* was found in the literature; this rarity is unfortunate given that meloxicam is among the most commonly used NSAID in laboratory animal medicine. In that study,⁴⁷ 10 mg/kg SC of meloxicam administered as a single injection immediately preceding intravenous inoculation of C57BL/C mice with syngeneic B16 melanoma cells significantly inhibited pulmonary seeding. Lung lesions were assayed by using fluorescent imaging on day 19. Ascertain the mechanism of action for this effect was beyond the scope of the study, but observations and analgesimetry suggested that pain was not a significant factor; therefore, the drug might have acted directly to inhibit the ability of cells to lodge in the lungs.

In other reports published over the past 20 y, several different NSAID administered by using an array of techniques have been examined. Celecoxib, a COX2-specific inhibitor, has received substantial attention and exerts dramatic effects on cancer progression. In a rat cornea angiogenesis model, celecoxib administered through daily gavage for 4 d resulted in a reduction in the area of neovascularization by 78.6%, a 2.5-fold increase in apoptosis, and a 65% decrease in endothelial cell proliferation.⁴³ In the same study, HT29 and HCT116 colon cancer xenografts were implanted into nude mice fed celecoxib in the diet to achieve a 25-mg/kg daily dose for the duration of the study.⁴³ HT29 tumor growth was reduced by 74% and HCT116 growth by 75%. Assays for blood vessel formation, apoptosis, and cell proliferation yielded results similar to those of the rat corneal angiogenesis experiments.⁴³ Of note, neither colon cancer cell line produced noteworthy amounts of COX2,⁴³ making it unlikely that direct inhibition of enzyme activity was responsible for the observed results.

In other studies of celecoxib, the agent administered in drinking water for 19 d to A/J mice significantly inhibited the growth of TA2–MTXR murine mammary tumor xenografts placed subcutaneously.⁶⁹ Treatment resulted in a 22.3% reduction in tumor volume and significantly reduced blood vessel counts both in the primary tumor and in metastatic lung lesions. Immunohistochemistry of primary tumors showed lower VEGF expression and cell proliferation and increased apoptosis after treatment. In the same study,⁶⁹ an *in vitro* assay using chick chorioallantoic membranes showed significant dose-dependent reduction in blood vessel formation. A similar investigation was conducted by using subcutaneous xenografts of PC3 prostate cancer cell lines implanted into nude mice.⁶³ Diets supplemented with 3 different concentrations of celecoxib that were fed from days 6 through 30 were found to reduce tumor volume by 26% to 52% in a dose-dependent manner. Immunohistochemistry revealed a 50% reduction in cell proliferation and microvessel density.⁶³ Lastly, celecoxib inhibited azoxymethane-induced colon tumors in male F344 rats. Those receiving celecoxib in the diet for 50 wk showed a tumor incidence rate of 6% compared with 85% in animals fed nonmedicated diet.³⁷ These reports show that COX2 inhibitors such as celecoxib possess substantial anticancer properties, acting primarily by inhibiting new blood vessel formation and cell proliferation while promoting apoptosis.

Another group of reports discusses the effects of a wide array of COX-selective and nonselective NSAID, some of which are purely for experimental purposes and unavailable as therapeutic compounds. Nude mice inoculated subcutaneously with the human ovarian cancer cell line SKOV3, which is known to express COX1, received either the COX1-specific agent SC560,

COX-nonspecific ibuprofen, or both.⁴⁵ The agents were administered for 21 d through oral gavage at 4 or 50 mg/kg daily, respectively and near the recommended analgesic dosage of 40 mg/kg for ibuprofen. SC560 exerted a stronger antitumor effect than ibuprofen, and combination therapy showed the greatest activity, resulting in a 41.55% reduction in tumor volume. Similarly, SC560 was more effective than ibuprofen at reducing VEGF transcription and angiogenesis; again, combining the 2 agents showed the greatest effect. In a separate study, SKOV3 cells were placed subcutaneously in SCID-beige mice treated with the nonselective NSAID sulindac or flurbiprofen alone or in combination in the diet for 8 wk.¹⁷ Single-agent or combination treatment resulted in 40% to 45% or 57% reduction in tumor volume, respectively. A selection of commonly used NSAID, including meloxicam, ibuprofen, and celecoxib, was tested in vitro for their abilities to induce apoptosis in 3 additional ovarian cancer cell lines (36M2, SW626, CAOV3). All agents induced cell death to various degrees in all cell types, and as with the animal studies, combination treatment regimens had more pronounced effects.¹⁷ Two very similar investigations were conducted by using 4T1 cells administered to BALB/C mice.^{13,68} The effects and mechanisms of action for the COX-2 specific SC 236 or nonselective indomethacin administered daily for 13 or 14 d were examined.^{13,68} Both studies reported reduced cellular proliferation and angiogenesis and increased apoptosis. In vitro assays similarly showed enhanced apoptosis as well as decreased VEGF production, correlating well with reduction in new blood vessel formation observed in vivo.¹³ A different group took a novel approach by dosing TRAMP (transgenic adenocarcinoma of the mouse prostate) mice with *R*-flurbiprofen, which is a single-enantiomer form of the drug that exerts no COX enzyme inhibition.⁸³ Treated mice showed a 19% reduction in primary adenocarcinoma incidence as well as a 34% reduction in metastasis, suggesting that NSAID can exert their effects through mechanisms other than COX inhibition, corroborating data from studies using cell lines that do not overexpress COX enzymes.⁶³

Completing the roster of reports on NSAID are several that explored the effects of aspirin; these studies are not surprising given the human literature suggesting the drug's usefulness as a chemopreventative. Data from multiple animal models support this thesis, all of which used nude mice and a variety of cancer cell types. Aspirin orally administered for 7 wk significantly reduced HepG2 hepatocellular carcinoma tumor weight and volume.³³ In vitro assays showed increased apoptosis through both extrinsic and intrinsic signaling pathways.³³ Aspirin likewise reduced MDA-MB-231 mammary adenocarcinoma tumor volume when given daily for 15 d starting from when a palpable tumor was first identified.⁵¹ This same study showed a preventative effect when the drug was started 10 d prior to inoculation, as evidenced by delayed onset of tumor growth.⁵¹ In vitro assays showed enhanced apoptosis, as well as reduced tumor cell migration and progrowth signaling, in the presence of aspirin. Lastly, aspirin administered through gavage for 14 d to animals implanted with RKO human colon adenocarcinoma cells resulted in a significant reduction in tumor weight and volume and increased apoptosis within tumor tissue.⁶⁴ In vitro assays showed downregulation of several genes involved in cell growth regulation and angiogenesis.⁶⁴

Considerations and Conclusions

The primary goal of the present review was to examine the current body of published literature addressing the influence of pain and analgesic agents on rodent models of cancer. The

hope is that this information will provide guidance to laboratory animal professionals, IACUC members, and researchers who share an obligation to ensure that both animal welfare and the scientific integrity of the model are duly considered. Sufficient evidence is available to support the idea that rodent cancer models likely engender some degree of pain that left unrelieved, serves as a confounding study variable, primarily by inducing a stress response. In addition, sufficient evidence exists to support the conclusion that analgesics influence cancer biology and likewise serve as confounding factors. Opioids exert mixed activities on cancers that are directly permissive but indirectly inhibitory, most likely through control of pain and immune modulation. NSAID consistently show anticancer effects, most likely through COX enzyme inhibition, receptor binding, and as-yet-undefined mechanisms, resulting in reduced cell proliferation and angiogenesis coincidental with increased apoptosis. Although many of the agents studied were those seldom used by laboratory animal professionals to manage pain, those that were used share the same central mechanisms of action. Given this caveat, the existing literature does provide useful insight, and it is reasonable to conclude that the administration of agents commonly used in pain management, such as meloxicam, carprofen, and buprenorphine, should be done with full acknowledgment that some effect on tumor growth is possible. Consideration of all of these potential and actual effects creates something of a 'Catch-22' situation, and any argument in favor of withholding analgesia because of concerns over the potential to confound results must be made with full acknowledgment that the converse might also be true.

To add further complexity, several routine manipulations and environmental conditions have been shown to influence tumor growth in animals. Anesthetic agents used for either surgery or restraint during noninvasive procedures such as imaging in addition to nonphysical stressors, have been shown to influence tumor growth in rodents. Dexmedetomidine at low doses exerts protumor effects.⁶ Ketamine, thiopental, and halothane suppress NK cell activity and promote metastasis, whereas propofol does not.⁵³ The stress response to any stimulus—whether physical or psychologic—has procancer effects. Mimicking the stress response through the administration of epinephrine and cortisol reduced survival in a rat model of leukemia.³⁴ Mice raised in social isolation showed increased colonic tumor growth compared with group-housed animals, and psychologic stress was concluded to be a primary factor in allowing for enhanced tumor growth.⁸⁵ Other groups who examined the role of psychologic stressors agree.⁶² It is easy to lose sight of these seemingly innocuous factors as attention focuses more squarely on questions of pain and analgesic use, but it would be foolish to ignore them.

The available data help to steer decisions, but a clear need for future investigation remains. Of particular benefit would be more studies of agents routinely used in laboratory animal medicine. Buprenorphine, meloxicam, and carprofen are underrepresented, and studies examining their effects on a variety of cancer models while taking robust measures of pain would be invaluable to the field. In addition, many studies have been performed in strains with fully intact immune systems by using syngeneic cancer cells, thereby leaving the opportunity for more investigations using nude, SCID, or nonobese diabetic (Nod)-SCID gamma (NSG) strains, for example, some of which lack multiple immune cell type populations. Given that pain stress and some analgesic agents, notably opioids, exert their effects through immune modulation, it is interesting to consider their influence on these models.

Because all of this information may seem overwhelming, returning to the core ethical obligation we have to relieve pain in our animal models is prudent. This charge should be the platform on which all discussions about withholding analgesia are built. Although we are occasionally left without robust evidence that pain exists, it might be prudent to embrace the precautionary principle and build treatment plans for the animals in our care on the presumption that pain does exist. By all means, in the face of supporting evidence that analgesic agent provision will invalidate or confound a study, withholding could be justified. In other words, thoughtful evaluation of the design, aims, and endpoints in every study by all who contribute to promoting animal welfare and good science is an important and necessary process.

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