Winter 2010
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WEILL CORNELL MEDICAL COLLEGE EDITION

In Focus

Center of Comparative Medicine & Pathology
Research Animal Resource Center
Laboratory of Comparative Pathology

CCMP Website

Introducing the Center of Comparative Medicine and Pathology

The rapid pace of scientific innovation has driven the development and use of animal models at WCMC, as well as at institutions worldwide. In particular, the development of techniques to manipulate the mouse genome has led to a marked increase in the use and value of mouse models of human disease, especially cancer. WCMC expects this trend to continue and will continue to make significant investments to meet the expanding need for animal models. The Center of Comparative Medicine and Pathology (CCMP) was created and serves as a major component of this expansion.

The CCMP is an academically oriented, interdisciplinary center focused on animal research support, training specialists in laboratory animal medicine as well as comparative and genomic pathology, and conducting translational and collaborative research. It serves as the home for the academic programs and services that support the development, characterization, care, and use of animal models at WCMC.

The Center's constituent components include the Research Animal Resource Center (RARC), which provides husbandry and clinical care services; the Laboratory of Comparative Pathology (LCP); and various pre- and postdoctoral training programs. Additional information on the CCMP and its training programs, as well as the disciplines of comparative medicine and pathology can be found on the CCMP's web site - http://weill.cornell.edu/research/rea_sup/comp_med/index.html

Pain Control in Rodents

Experimental Considerations in the Selection of an Analgesic for Pain Control

Pain adversely impacts the welfare of animals used in research and, if not controlled, can introduce a variable that can confound the interpretation of data. Regulations mandate that procedures that are expected to result in pain or distress in humans be assumed to have the same effect on animals and pain relief must be provided, unless scientifically justified and approved by the IACUC.

The potential impact on research is a concern when evaluating analgesic choices. Their effects on the immune system may affect several areas of research. Buprenorphine (Buprenex®), a commonly used analgesic in rodents, is frequently employed for procedures or conditions that can be expected to result in moderate to severe pain. There is concern regarding the impact of this analgesic on the immune system, as pure mu agonist opioids, such as morphine, can affect the immune system. Morphine alters innate and adaptive immunity, and reduces cellular immunity. Buprenorphine, also an opioid, is a partial agonist at the mu receptor. It is more potent than morphine and has a desirable (for clinical use in rodents) longer duration of action.

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Phytoestrogens in Rodent Diets, Part II: Their Potential Impact on Science

Phytoestrogens are structurally similar to endogenous estrogens and are found in plant materials commonly used in laboratory rodent diets. Soy and alfalfa are two such constituents that contain measureable amounts of phytoestrogens that act as selective estrogen receptor modulators. Phytoestrogens may induce potent and variable effects on cancer research. Countries where people consume relatively higher amounts of dietary soy appear to have a lower incidence of breast, prostate and colon cancer compared to cultures that consume lesser amounts. Isoflavones are one of the components in soy that is proposed to induce anticancer effects, but other factors including protease inhibitors, phenolic compounds and methionine deficiency have also been implicated. A number of studies have shown that diets with higher levels of phytoestrogens reduce the histological appearance of neoplasms, reduce the number of tumors, and/or delay tumor development. Some contradictory studies, however, have indicated that rats on diets containing genistein fail to prevent mammary tumor regression after ovariectomy and may promote continued

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http://www.menopausematters.co.uk/phytoestrogens.php

Phytoestrogens- friend or foe?

Virginia Gillespie, DVM, the LCP's first fellow in Comparative and Genomic Pathology.

Important Notice:
KETAMINE RECALL!
Watch for a broadcast message on this subject. Contact Veterinary Services or EQA for details.

Fellowship in Comparative and Genomic Pathology Admits its First Fellow

With the rapid increase in the development and use of animal models of human disease during the past two decades, particularly the ability to genetically engineer specific models, there has been a corresponding increased demand for pathologists trained in comparative pathology. Comparative pathologists have the skills to interpret changes at the organ, tissue, and cellular levels and understand the human disease for which the model was developed.

Because of its extensive resources and the large variety of distinct genetically engineered rodents that it evaluates, the Laboratory of Comparative Pathology (LCP), which supports scientists at Memorial Sloan-Kettering Cancer Center, Weil Cornell Medical College and The Rockefeller University, provides an ideal training environment for veterinarians and physicians who have completed training in general anatomic pathology and desire specialized training evaluating animal models and the molecular basis of human disease. Working under the guidance of three board-certified veterinary comparative pathologists in the LCP, fellows are exposed to a variety of models and disease processes. They also interact (through various rounds and other training sessions) with human pathologists at the Tri-Institutes, further bridging the gap between veterinary and human pathology.

Virginia Gillespie, DVM (see inset) is the first fellow participating in the program. Dr. Gillespie obtained her Doctorate in Veterinary Medicine degree from the University of Wisconsin School Of Veterinary Medicine, then completed 3 years of general anatomic pathology training in the Quad-Institutional Training Program in Veterinary Pathology before beginning her fellowship.

Rodent Surgery Guidelines- Recent Updates

In October 2009, the IACUC Rodent Survival Surgery Guidelines were revised and several important changes were instituted.

There is a new version of the blue "Surgery Card" and two new forms, one for post-operative monitoring one for intra-operative. The new card is to be used following all survival surgical procedures but the post-operative monitoring form is required is select cases only and the intra-operative form is purely voluntary.

RARC's Education & Quality Assurance (EQA), service staff is available to meet with laboratory staff at your convenience to review the new requirements and assist the staff in implementing these changes. In addition we have prepared a short PowerPoint presentation highlighting the changes which will be available on the RARC website.

Update on Storage and Dosing of Ketamine and Ketamine Combinations

As a result of recent studies, RARC has revised storage guidelines and dosage recommendations for the Ketamine-Xylazine Anesthetic Cocktail. The expiration date of the cocktail is now **6 months from the date of preparation,** or the expiration date of either anesthetic component, whichever comes first. Both Ketamine and Ketamine-Xylazine cocktails must be stored in a controlled substance drug lock box, not in a refrigerator.

Finally, the recommended dose for surgical anesthesia in mice has been **increased to 150mg/kg Ketamine and 15 mg/kg Xylazine**. Please contact Veterinary Services or Education and Quality Assurance staff for further details.

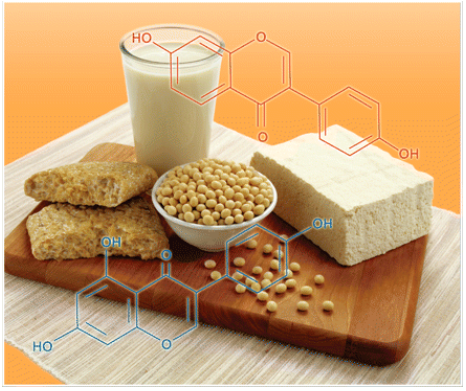
Reference :

Taylor BJ, Orr SA, Chapman JL, Fisher DE. 2009. Beyond-use dating of extemporaneously compounded ketamine, acepromazine, and xylazine: safety, stability, and efficacy over time. J Am Assoc Lab Anim Sci 48(6):718-26

Phytoestrogens in Rodent Diets, Part II

http://images.google.com/imgres?imgurl=http://biology.clc.uc.edu/graphics/taxonomy/plants/spermatophyta/

Red clover-an herb rich in phytoestrogens



caonline.amcancersoc.org/.../issue5/cover.shtml

Common soy-based foods and structural formulas of soy isoflavones genistein (lower, in blue) and daidzein (upper, in red).

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Other studies evaluating genistein, daidzein and isoflavones have shown that when these components are present in the diet there is a reduction in prostate carcinogenesis. As with effects of phytoestrogens on the reproductive system, effects on tumor development are variable depending on a variety of animal, dietary and endpoint factors.

Unlike the reproductive and tumor effects due to phytoestrogens, the effects on bone metabolism have been highly consistent. Soy isoflavones have been shown to have bone-sparing effects in postmenopausal women and ovariectomized rodents. It has been shown that 1000ug/100g body weight of daidzein for 90 days prevented bone loss in ovariectomized rats. Research has indicated that isoflavone levels above 300 µg/g feed will reduce bone loss and may interfere in studies on bone loss, therefore, researchers evaluating bone loss should be aware of dietary phytoestrogen levels.

Phytoestrogens may also impact behavioral research due to estrogen receptors located within the central nervous system. These estrogen receptors play a role in behavioral alterations, stress and brain biochemistry. Research on the influence of phytoestrogens in stress, anxiety and pain models has shown that phytoestrogens, including isoflavone, can suppress neuropathic pain responses. Rats may show reduced or elevated anxiety in elevated plus mazes depending upon study design. Rats consuming diets containing 600 µg/g diet of isoflavones were shown to have significantly elevated ACTH. In another study, rats on phytoestrogen diets spent less time in social interactions than rats on a phytoestrogen-free diet. In this study both groups of rats had similar levels of corticosterone, but rats on the isoflavone diet had higher stress-induced levels of the hormone. Alterations in physiological responses due to phytoestrogens may have serious implications in behavioral and hormonal studies.

They are known to lower blood cholesterol, especially LDL and VLDL, in animals and are therefore an important consideration in cardiovascular research. Male rhesus macaques fed a high fat diet supplemented with soy flour had a 30% reduction in serum LDL and VLDL compared to controls. Gerbils fed isoflavones had altered expression of lipid related genes and rats on a soy diet had altered cholesterol and fatty acid metabolism by liver enzymes. It has also been shown that genistein is a potent inhibitor of protein tyrosine kinases, which are needed for the binding of some growth factors to their receptors. These growth factors may promote the growth of plaques in arthrosclerosis indicating another cardioprotective role of phytoestrogens that may serve as a confounding variable in research.

The concentration of phytoestrogens in standard rodent chows varies between batches depending on plant factors, and a given concentration of phytoestrogen may have variable responses due to a variety of animal factors. In addition, study design is an important consideration when dietary phytoestrogen levels may have confounding or disruptive research effects. When comparing current results with previously published data, it is important that animal models are standardized and reproducible in order to minimize variability between studies and to accurately interpret research data.

References

1. Brown, N.M. and Setchell K.D.R. Animal models impacted by phytoestrogens in commercial chow: implications for pathways influenced by hormones. Laboratory Investigations. Vol. 81(5): 735-47. 2001.
2. Jensen, M.N. and Ritskes-Hoitingna, M. How isoflavone levels in common rodent diets can interfere with the value of animal models and with experimental results. Laboratory Animals. Vol. 41: 1-18. 2007.
3. Naciff, J.M., Overmann, G.J., Torontali, S.M., Carr, G.J, Tiesman, J.P. and Daston G.P. Impact of the phytoestrogen content of the reproductive system in the immature female rat. Environmental Health Perspectives. Vol 112(15): 1519-26. 2004.
4. Naz, R.K. Endocrine Disruptors: Effects on Male and Female Reproductive Systems. H.B. and Whitten, P.L. Chapter 4: Dietary Phytoestrogens Patisaul. CRC Press: Washington DC, 1999.
5. Purina labdiet. Phytoestrogens: fact, fiction, and the future. http://www.labdiet.com/phytoestrogens.htm

Experimental Considerations in the Selection of an Analgesic for Pain Control

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Morphine and fentanyl have been shown to stimulate the hypothalamic-pituitary-adrenal (HPA) axis, decrease natural killer (NK) cell activity, and augment tumor metastasis in rodents. In a study that used a low dose of buprenorphine (0.1mg/kg) in rats, these alterations were absent (Franchi, S. et al, 2007). However in a study using escalating doses of buprenorphine in rats, a dose-dependent suppression of splenic NK cell activity, lymphocyte proliferation and IFN-gamma production was reported (Carrigan, KA, et al, 2004). A similar study in the mouse compared the effects of buprenorphine (5 mg/kg; a dose considerably higher than used clinically in rodents) and fentanyl on splenic cellular immune responses. Parameters evaluated included lymphoproliferation, natural killer cell activity, and interleukin-2 and interferon-gamma production. In contrast to the immune alterations noted after administration of the pure mu agonist fentanyl, no alterations were observed in mice receiving buprenorphine (Martucci, C. et al, 2004). D'Elia, M., et al (2003) noted that buprenorphine did not modulate total CD 4+ or CD8+ splenic and thymic populations, but splenocytes from mice in this study that were exposed to buprenorphine for 5 days via an osmotic pump, exhibited greater proliferation upon anti-TCR monoclonal antibody stimulation than control mice.

It is important to remember that the performance of a surgical procedure or induction of disease can each result in significant immune alterations. In rats, surgical stress is associated with increased corticosterone levels, decreased NK cell activity and enhancement of tumor metastasis of NK sensitive tumors. Sacerdote, P. et al. (2006) reported that, when rats received buprenorphine, fentanyl, or morphine prior to an experimental surgery, only buprenoprhine prevented surgically induced immunosuppression. In fact in rats, buprenorphine has been demonstrated to reduce adrenocorticotropic hormone (ACTH) and corticosterone plasma levels, without altering either the HPA axis or measures of immune system function (Gomez-Flores, R and Weber, RJ, 2000). Therefore the use of analgesics should be viewed as a method to decrease variability by reducing physiologic and immunologic responses as a result of pain, rather than serving as a source of variability.

For both ethical and scientific reasons, analgesia should be administered prior to the painful insult. If a painful procedure is performed on all animals and analgesia is only administered to those animals that demonstrate pain, treatment is not uniform which may result in an unwanted experimental variable. It is advisable to perform a pilot study if there are concerns regarding the impact of an analgesic on your study. If post-procedure pain is expected, an analgesic such as buprenorphine can be administered to both sham and experimental groups. The immunomodulatory effects of buprenorphine can be compared with a non-steroidal anti-inflammatory agent, such as carprofen, and the analgesic that does not interfere with measured values is chosen.

In summary, there are conflicting reports in the literature regarding the effect of buprenorphine on the immune system in mice and rats. There is much evidence, however, to demonstrate that when compared to pure mu agonists, the partial agonist buprenorphine results in less immunomodulation. With all research, an attempt is made to minimize variability. Pain and the resulting responses are variables which can be readily controlled; however, the method in which it is controlled requires special attention when designing experiments.

References:
1. Carrigan KA, Saurer TB, James SG, Lysle DT. 2004. Buprenorphine produces naltrexone reversible alterations of immune status. Int Immunopharmacol. 4(3):419-28.
2. D'Elia M, Patenaude J, Hamelin C, Garrel DR, Bernier J. No detrimental effect from chronic exposure to buprenorphine on corticosteroid-binding globulin and corticosensitive immune parameters. 2003. Clin Immunol. 109(2):179-87.
3. Franchi S, Panerai AE, Sacerdote P. 2007. Buprenorphine ameliorates the effect of surgery on hypothalamus-pituitary-adrenal axis, natural killer cell activity and metastatic colonization in rats in comparison with morphine or fentanyl treatment. Brain Behav Immun 21(6):767-74. Epub 2007 Feb 8.
4. Gomez-Flores R, Weber RJ. 2000. Differential effects of buprenorphine and morphine on immune and neuroendocrine functions following acute administration in the rat mesencephalon periaqueductal gray. Immunopharmacology 48(2):145-56.
5. Martucci C, Panerai AE, Sacerdote P. 2004. Chronic fentanyl or buprenorphine infusion in the mouse: similar analgesic profile but different effects on immune responses. Pain 110(1-2):385-92.
6. Sacerdote P. 2006. Opioids and the immune system. Palliat Med 20 Suppl 1:s9-15.

UPCOMING SEMINARS

ESTABLISHING HUMANE ENDPOINTS FOR LABORATORY RODENTS

Lee-Ronn Paluch, BVSc, Post-Doctoral Fellow
Tri-Institutional Training Program in Laboratory Animal Medicine & Science
Place: MSKCC, RRL, Room 116
Date:Wednesday, February 17
Time: 2:00 - 3:30

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WHAT A DIFFERENCE A LAB MAKES! INTRODUCTION TO THE LABORATORY OF COMPARATIVE PATHOLOGY & the GENETICALLY ENGINEERED MOUSE PHENOTYPING SERVICE

Linda Johnson, DVM, MS, MPH, DACVP; Head, LCP's Section of Anatomic Pathology and the Genetically Engineered Mouse Phenotyping Service
Place: WCMC, Room E-701
Date: Wednesday, April 21
Time: 2:00 - 3:30