Summer 2009
Volume 2, Issue 2

WEILL CORNELL MEDICAL COLLEGE

In Focus

Research Animal Resource Center

New Professional Staff Join RARC

Christine Lieggi, DVM, DACLAM
Associate Director, Head of RARC's Veterinary Services

Linda Johnson, DVM, DACVP
Head, LCP's Anatomic Pathology Section and GEM Phenotyping Service

Dr. Christine Lieggi has joined RARC as Associate Director and Head of Veterinary Services. Dr. Lieggi recently relocated from Abu Dhabi, UAE where she worked for the American Veterinary Clinic. She received her veterinary degree from Michigan State University and completed a postdoctoral fellowship in laboratory animal medicine at the University of Illinois at Chicago (UIC). She has been a Diplomate of the American College of Laboratory Animal Medicine since 2005. Christine has experience with a wide range of animal species in the biomedical research setting having previously overseen the large animal clinical and animal surgical programs at UIC. At WCMC, Dr. Lieggi oversees all of RARC's clinical veterinary programs and is actively involved in the Center's developing aquatic models program.

Dr. Linda Johnson, an American College of Veterinary Pathology-board certified veterinary pathologist with over 20 years of experience, recently assumed the leadership of the Laboratory of Comparative Pathology's anatomic pathology section and genetically engineered mouse phenotyping service. A graduate of Kansas State University's College of Veterinary Medicine, Dr. Johnson completed a fellowship in comparative pathology at Johns Hopkins University School of Medicine. Subsequently, she has served as a comparative pathologist on 3 continents in various positions which included posts at the Armed Forces Institute of Pathology, Albert Einstein College of Medicine, Yale University School of Medicine as well as a consultant pathologist to the British government at the Veterinary Laboratories Agency.

Inside Interest:

Phytoestrogens in Rodent Diets: A Confounding Research Variable

Edamane

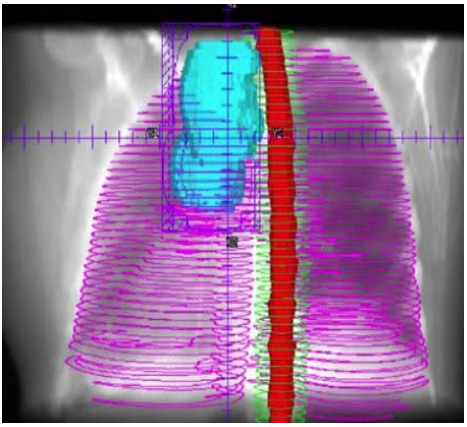
Phytoestrogens are diphenols that bear structural similarities to endogenous estrogens and are commonly found in plant materials used to formulate laboratory rodent diets. Soy and alfalfa are two such ingredients in commercial rodent diets that contain measureable amounts of phytoestrogens.

Phytoestrogens act as both agonists and antagonists at select estrogen receptors. This property results in phytoestrogens causing different physiologic effects influencing a variety of body systems, including the reproductive, skeletal, central nervous, and the cardiovascular, as well as tumor development.

The two major classes of phytoestrogens are the lignans and the isoflavonoids. The lignans are a major component of the cell wall of plants. Isoflavonoids are less prevalent than lignans, but are found in high concentrations in soybeans & soybean products. Isoflavonoids can also be present in whole grains, potatoes, fruits and vegetables. Isoflavonoids can be further subdivided into isoflavones, isoflavans and coumestans. Genistein and daidzein are the two most common isoflavones found in soy. The most potent phytoestrogen is the coumestan, coumestrol, which is found in highest concentrations in alfalfa. Both soy and alfalfa can be constituents of animal diets. The concentration of phytoestrogens detected within an animal depends on plant as well as animal factors. The effects of varying biological levels of phytoestorgens may have a confounding influence on animal research.

Cont. on pg. 3

The Use of Heparin to Prevent Sequelae in a Mouse Model of Bone Metastasis



Non-Small Cell Lung Tumor

Bone metastasis is common with certain cancers in humans, including breast, prostate, lung, kidney, and thyroid. Animal models of bone metastasis are utilized to help understand tumor pathogenesis and evaluate potential therapeutics, but the event is rare in spontaneous neoplasms of laboratory and domestic animal species. Therefore, a variety of techniques have been employed to increase the incidence of bone metastasis with certain human tumor cell lines in animal models of cancer.

Techniques that increase bone metastasis in mice include orthotopic injection, typically into the mammary fat pad or prostate, intracardiac injection into the left ventricle, and intraosseous injection of tumor cells. Inoculation of tumor cells into the left ventricle allows the cells to bypass the pulmonary vasculature and be distributed to organs with blood flow.

Intracardiac injection increases bone metastasis in mice at an earlier stage of disease. The intracardiac tumor injection technique has primarily focused on breast (eg, MDA-MB-231) and prostate (eg, PC3) models. Lung carcinomas also metastasize to bone in humans, necessitating the need for a mouse model.

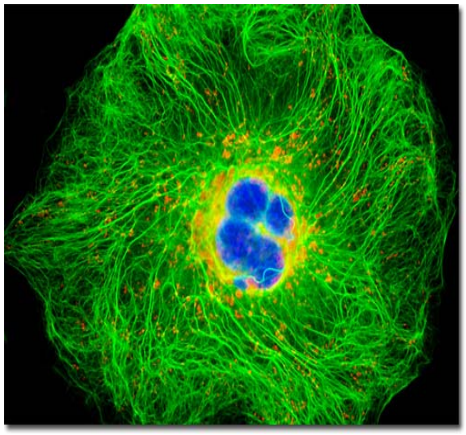
Stocking and colleagues (Comp Med. 2009: 59(1): 37-45.) have demonstrated that complications which can accompany the intracardiac injection technique can be prevented by using low-molecular-weight heparin (enoxaparin). They describe stroke-like signs, including head tilt, spinning, failure to recover from anesthesia, and high mortality in athymic nude mice following intracardiac injection of non-small cell lung carcinoma cells. They hypothesized that the clinical signs and mortality were due to thromboembolism formation post-intracardiac injection, supported by the procoagulant activity of tumor cells in general.

These authors evaluated the development of a hypercoagulable state post-intracardiac injection, the ability of a low-molecular-weight heparin (enoxaparin) to prevent this state, and the effect of enoxaparin on mouse survival and tumor metastasis. Hypercoagulability post-intracardiac injection was evaluated by coagulation assays and histologic analysis of tissues for thromboembolism formation.

Mice that received 10 mg/kg enoxaparin IV, ten minutes prior to intracardiac injection of 1 x 106 H2126luc non-small cell carcinoma cells survived and did not develop a hypercoagulable state nor were thromboemboli apparent histologically. Mice not pretreated with enoxaparin died within five to ten minutes post-intracardiac injection and exhibited histologic evidence of a hypercoagulable state characterized by numerous pulmonary, brain and renal thromboemboli. Although coagulation assays were not successfully performed on animals from this group, complete blood chemistry revealed decreased platelets consistent with consumption.

Enoxaparin's effect on long-term mouse survival and tumor metastases was also evaluated with two non-small cell lung cancer lines (H2126luc and H1975luc). Administering 10 mg/kg enoxaparin ten minutes prior to intracardiac tumor challenge decreased mortality without altering tumor burden or metastasis. Tumor burden and metastases were evaluated with bioluminescent imaging, radiography, and histology and no significant differences were noted from the control group.

Mousa and colleagues (Thromb Haemost. 2006; 96: 816-21.) showed that mice treated with 10 mg/kg enoxaparin IV daily for 14 days had reduced metastatic tumor burden when using B16F10 murine malignant melanoma cells, but this effect was not observed by Stocking and colleagues who utilized a shorter treatment course. Long-term survival was unaffected by the enoxaparin pretreatment as tumor growth progressed as expected.



Human Lung Carcinoma Cell (A-549)~ Fluorescence Digital Image Gallery

http://www.olympusmicro.com/copyright/index.html

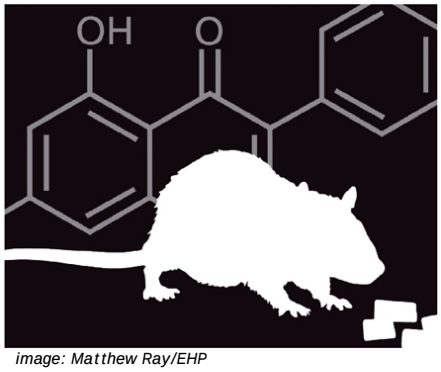
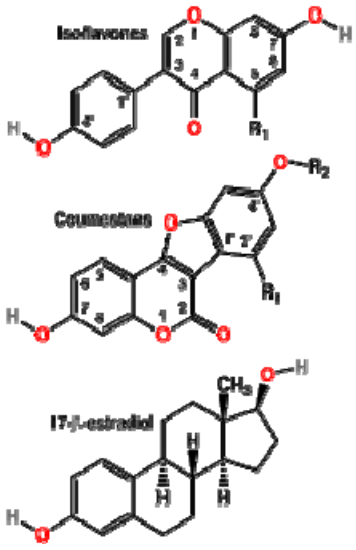


image: Matthew Ray/EHP



Chemical structures of the most common phytoestrogens found in plants (top and middle) compared with estrogen (bottom) found in animals.

Phytoestrogens in Rodent Diets

cont. from Pg. 1

The amount of phytoestrogens in a given crop is dependent on a variety of factors related to growth and handling of the crop. Soil quality parameters, temperature, humidity, season, day length, time of harvest as well as other conditions vary annually. Post-harvest processing of the crop may also influence phytoestrogen levels. Therefore, most food stuffs vary in phytoestrogen content from batch to batch except for chemically defined rodent diets that have almost no variability between batches but lack palatability.

It is also recognized that plasma concentration of lignans and isoflavonoids differ among groups of humans depending on dietary choices. Vegetarians and persons of Asian descent, who consume relatively higher quantities of soy, have higher plasma levels of lignans & isoflavonoids compared to omnivores and people of Western descent.

Plant and dietary factors determine the potency of phytoestrogens in a feed stuff and therefore the level of phytoestrogens consumed by an animal. After an animal consumes a diet containing phytoestrogens, numerous factors determine the effect of these phytoestrogens once they accumulate within the body. Differences between animals may be due to differences in species, strain, sex, as well as the timing and duration of phytoestrogen exposure. Body size and fat composition alter plasma and urine concentration of phytoestrogens due to storage in various tissue compartments. Phytoestrogens are excreted in all body fluids including urine, feces, blood, bile, and plasma. Timing of exposure to phytoestrogens has variable effects on an animal during different life stages (gestation, neonate, weanling, adult) as well as the total length of exposure. All of these factors must be considered when determining the significance of phytoestrogens in rodent feed.

The selection of endpoints is another factor to consider when determining the role of phytoestrogens in research. Endpoints that are sensitive to estrogenic or anti-estrogenic effects such as reproductive organ weight, hormone levels, and anogenital distance may be affected by dietary phytoestrogens. Researchers that monitor these endpoints need to be cognizant of phytoestrogen levels in animal feed and its potential influence on biological processes.

The influences of dietary factors, route of administration (supplementation, force feeding, various diets, etc.), animal factors and study design have resulted in many inconsistencies regarding the effects of phytoestrogens on the reproductive tract of laboratory rodents. The Uterotropic and the Hershberger Assays are used to assess endocrine disruption in rats and may be influenced by varying levels of phytoestrogens in the diet. Depending on study design, dietary isoflavones may cause an increase, decrease or no change in uterine weight. Studies utilizing male rats provided dietary phytoestrogen concentrations ranging from 300-920 µg/g diet demonstrated variable signs including reduced serum testosterone and androstenedione, reduced androgen responsiveness, reduced anogenital distance, and smaller testes, epididymi and prostate glands. Studies that rely on reproductive parameters should be carefully designed to consider the effect of phytoestrogens in the diet.

PHYTOESTROGEN IN RODENT DIETS, Part II, in the next InFocus will explore the effects on cancer, behavior and physiology research

Animal Welfare Concerns

WCMC is committed to the humane care and use of animals in research, teaching and testing. The use of animals is a privilege based on the public's trust and carries with it accountability to follow applicable state and federal laws and regulations as well institutional policies.

To that end, it is essential that WCMC provides a mechanism for reporting concerns regarding animal care and use. If you have information with respect to inappropriate animal care or use, such as inhumane treatment, unsuitable housing conditions, and/or conducting procedures not approved by the IACUC, there is a moral and ethical imperative to report such activities.

If any WCMC employee has questions or concerns regarding the humane treatment of animals or if they suspect misuse or abuse they are strongly encouraged to make a confidential report by contacting the IACUC Chair or Vice-Chair, RARC's Director, or WCMC's Compliance Officer.

A thorough investigation will be conducted and all reports will be held in strict confidence. No employee will be subject to discrimination or reprisal for reporting such concerns.

September ~ November
Seminars

All are welcomed to attend!

UPCOMING SEMINARS

ANIMAL MODELS OF CANCER
Christine Lieggi, DVM, DACLAM
Assoc. Director, RARC Head of Veterinary Services
Place: MSKCC, ZRC, Room 105
Date: Wednesday, September 16
Time: 2:00 - 3:30

WHAT DO IACUC'S WANT?
Andrew Nicholson, DVM, Ph.D., Director, Animal Research Protections Program, WCMC/MSKCC Place: MSKCC, RRL, Room 101
Date: Wednesday, October 21
Time: 2:00 - 3:30

From ANOVA to 3Rs : Path to Research Excellence
Engin Ozertugrul, BS, MS, LATG
IACUC Coordinator, CBC
Place: Rockefeller University, Weiss 302
Date: Wednesday, November 4
Time: 2:00 - 3:30 PM

WCMC TRAINING SESSIONS: SEPT. - NOV. 2009

RARC Orientation
Tuesday, 9/1/09, 2:00-4:00
Thursday, 9/17/09, 10:00-12:00
Tuesday, 10/6/09, 2:00-4:00
Thursday, 10/15/09, 10:00-12:00
Tuesday, 11/03/09, 2:00-4:00
Thursday, 11/19/09, 10:00-12:00

Xenograft Training
Monday, 9/21/09, 2:30-3:30
Monday, 10/19/09, 2:30-3:30
Monday, 11/16/09, 2:30-3:30

Hazardous Materials Training
Wednesday, 9/23/09, 2:30-3:30
Wednesday, 10/28/09, 2:30-3:30
Wednesday, 11/18/09, 2:30-3:30

Rodent Surgery
Friday, 9/4/09, 10:00-11:30, wet lab
Monday, 9/21/09, 10:30-11:30
Friday, 10/2/09, 10:00-11:30, wet lab
Monday, 10/19/09, 10:30-11:30
Friday, 11/06/09, 10:00-11:30, wet lab
Monday, 11/16/09, 10:30-11:30

Rodent Breeding
Wednesday, 10/28/09, 10:00-11:30

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