The 16th Annual International Dose-Response Conference

PRECONDITIONING: IN BIOLOGY AND MEDICINE

MECHANISMS AND TRANSLATIONAL RESEARCH

The Annual Meeting of the International Dose-Response Society

ABSTRACT BOOK

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Hormetins as Drugs for Healthy Aging

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Physical, nutritional and mental hormetins initiate cellular stress responses and strengthen the homeodynamics for health and survival. Exercise, heat and irradiation are examples of physical hormetins, which activate heat shock-, DNA repair- and anti-oxidative-responses. Several non-nutritional chemical components in the food, such as flavonoids and polyphenols present in spices, herbs and other sources, are examples of nutritional hormetins, which induce anti-oxidative and anti-inflammatory stress responses. Calorie restriction and intermittent fasting are also hormetins, which activate the autophagic and sirtuin-mediated stress responses. Intense brain activity and focussed attention comprise mental hormetins, which also induce various stress responses. A successful strategy for discovering novel hormetins involves screening for multiple stress responses of normal cells in culture. This requires establishing immediate and delayed stress response profiles, followed by cell type-specific functional assays, after exposure to natural or synthetic compounds and mixtures. A combination of different hormetins can potentially be the drugs for maintaining, improving and recovering health during aging.

H2S-Based Anti-inflammatory Drugs: Lost and Found in Translation

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There is a rapidly expanding body of evidence for important roles of hydrogen sulfide in protecting against tissue injury, reducing inflammation, and promoting repair. There is also growing evidence that H_2S can be successfully exploited in drug development. H_2S synthesis and degradation are regulated in circumstances of inflammation and injury so as to promote repair and re-establish homeostasis. In animal studies, several novel H_2S-releasing drugs exhibited enhanced anti-inflammatory, analgesic and pro-restorative effects, while having reduced adverse effects in many tissues. H_2S is a pleiotropic mediator, having effects on many elements in the inflammatory cascade and promoting the resolution of inflammation and injury. It also contributes significantly to mucosal defence in the gastrointestinal tract, and in host defence against infection. The gastrointestinal microbiota is both a significant source and target of H_2S. A better understanding of the physiological and pathophysiological roles of H_2S continues to be restrained by the lack of simple, reliable methods for measurement of H_2S synthesis, and the paucity of highly selective inhibitors of enzymes that participate in endogenous H_2S synthesis. On the other hand, there is emerging evidence that novel, H_2S-based therapeutics are safe and effective in humans. One such drug, ATB-346 (an H_2S-releasing derivative of naproxen) is now in Phase 2 clinical trials for arthritis.

Preconditioning in Cardiology Applications

Remote Ischemic Preconditioning: From Bench to Bedside

Robert A. Kloner MD, PhD, Huntington Medical Research Institutes, Pasadena, CA, and Keck School of Medicine at the University of Southern California, Los Angeles, CA.

Ethanol Ingestion Elicits An Anti-inflammatory Phenotype To Limit Ischemia/Reperfusion Injury By A Neutrophil-Dependent Mechanism.

Ronald J. Korthuis, Department of Medical Pharmacology and Physiology and the Dalton Cardiovascular Research Center, University of Missouri School of Medicine, Columbia, MO

Ischaemic Preconditioning To Enhance Sport Performance: Waste of Time or No Time to Waste?

Dick H.J. Thijssen, Research Institute for Sport and Exercise Science, Liverpool John Moores University (UK) and Department of Physiology, Radboud University Medical Centre (The Netherlands)

Glutamate Dehydrogenase Is the Source of Signaling ROS Activating Cardioprotective Signaling Pathways During Ischemic Pre-Conditioning

James N. Weiss, MD, Guillaume Calmettes PhD, Scott John PhD, and Paavo Korge PhD, David Geffen School of Medicine at UCLA, Los Angeles, CA

Remote Ischemic Preconditioning: From Bench to Bedside

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The concept of remote ischemic preconditioning (RIPC) was first described by Dr. Karin Przyklenk et al in our laboratory of the Heart Institute of Good Samaritan Hospital in Los Angeles, in 1993. She showed that brief episodes of coronary artery occlusion followed by reperfusion of the circumflex coronary artery reduced myocardial infarct size induced by a more prolonged coronary artery occlusion of the left anterior descending coronary bed in an anesthetized canine model. Subsequently, Birnbaum in our laboratory induced brief episodes of lower limb ischemia in the rabbit and demonstrated that this conditioning at a distance reduced myocardial infarct size. There have been numerous experimental studies showing that remote ischemic conditioning (preconditioning, post conditioning, and preconditioning) is capable of protecting distant organs from ischemic necrosis. The mechanisms may include release of a humoral substance, a neural mechanism involving reflexes, and there is recent interest in exosomes and mRNA. There are now at least five clinical trials in the literature showing a benefit of remote conditioning in patients experiencing an acute myocardial infarction. Botker et al. utilized blood pressure cuffs to induce brief brachial artery occlusions and reperfusion of the upper arm starting in the ambulance in patients with acute myocardial infarction. Remote conditioning increased myocardial salvage index and improved long-term clinical outcomes. Remote conditioning has not been consistently positive in other settings such as cardiac surgery; however, these patients are already subject to cardio protection including use of hypothermia, cardiologic agents and cardio protective anesthetic agents. RIPC has shown promise in athletes and we are currently investigating it in a hemorrhagic shock model.

Ethanol Ingestion Elicits An Anti-inflammatory Phenotype To Limit Ischemia/Reperfusion Injury By A Neutrophil-Dependent Mechanism

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The aim of this presentation is to summarize a large volume of work indicating that antecedent ethanol ingestion at low to moderate levels induces the development of an anti-inflammatory phenotype that limits the extent of postischemic injury when tissues are exposed to ischemia/reperfusion (I/R) 24 hrs later, with a particular emphasis on the role of neutrophils in eliciting this protection. Work conducted in our laboratory and by others indicates that the anti-inflammatory and tissue-sparing effects of ethanol ingestion 24 hrs prior to I/R are triggered by transient-receptor potential vanilloid-1 (TRPV1) channel-dependent release of calcitonin gene-related peptide (CGRP) from capsaicin-sensitive sensory neurons. CGRP release is associated with the production of tumor necrosis factor alpha (TNFα). These proinflammatory mediators elicit the release and activation of matrix metalloproteinase-9 (MMP-9, and perhaps other proteases) from tissue resident neutrophils. The enzymatic activity of MMP-9 liberates matricryptins from the extracellular matrix. These proteolytic digestion products activate large conductance, calcium-activated potassium (BKCa) channels on endothelial cells, which in turn upregulate heme oxygenase-1 (HO-1) expression and activity by an Nrf2/ARE-dependent mechanism. The enzymatic activity of HO-1 produces the powerful antioxidants bilirubin and secondarily-derived biliverdin and the antiadhesive signaling molecule carbon monoxide, which act in concert to prevent postischemic expression of endothelial cell adhesion molecules, thereby limiting I/R-induced leukocyte infiltration and neutrophil-dependent tissue injury. The antioxidant actions of ethanol-induced HO-1 activity may also serve to protect soluble guanylyl cyclase from redox inactivation during reperfusion, thereby preserving arteriolar responses to endothelium-dependent vasodilators and limiting capillary no-relow. This work was supported by grants from the National Institutes of Health (AA-014945 and AA-022108).

Ischaemic Preconditioning To Enhance Sport Performance: Waste of Time or No Time to Waste?

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The clinical relevance of ischaemic preconditioning (IPC) may go beyond the traditional research areas of cardiology and vascular surgery. In the research area of sport and exercise science, studies show that enhanced blood supply (or delivery of blood to the periphery) and/or resistance against hypoxia is able to improve exercise performance. Exposure to IPC 'shares' the ability to increase blood flow and improve resistance against hypoxia. Consequently, recent studies have explored whether application of IPC can improve exercise performance *per se* and whether improvements occur via similar mechanisms. Therefore, the potential of IPC to enhance exercise performance will be discussed, with specific attention to factors that may alter the potency of IPC. Furthermore, exercise (training) and IPC both share the ability to protect against cardiovascular events. Recent studies in animals and humans have therefore explored the potential preconditioning effects of exercise. An overview of the potential preconditioning effects of exercise will be provided, with a discussion on how this knowledge can be applied in clinical practice, and how this helps to understand the cardioprotective effects of regular exercise training.

Glutamate Dehydrogenase Is the Source of Signaling ROS Activating Cardioprotective Signaling Pathways During Ischemic Pre-Conditioning

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One of the most powerful means known to protect the heart from ischemia/reperfusion (I/R) injury is by ischemic pre-conditioning (IPC). Its ability to protect the heart depends on the generation of signaling reaction oxygen species (ROS) by the brief pre-conditioning I/R episodes, which activate cardioprotection through the Reperfusion Injury Survival Kinase (RISK) and/or Survival Activating Factor Enhancing (SAFE) pathways. The origin of the signaling ROS generated during IPC is unclear, since electron transport complexes should not be damaged by the brief pre-conditioning I/R episodes and the $NADH/NAD^+$ ratio remains too low for most matrix dehydrogenases to generate significant ROS. The matrix enzyme glutamate dehydrogenase (GDH) is an exception. We show that when activated by ADP or GDP, purified GDH in vitro was capable of generating ROS from glutamate oxidation when supplied with NAD^+ only i.e. at a low $NADH/NAD^+$ ratio. Glutamate-dependent ROS production could also be demonstrated in isolated cardiac mitochondria from wild-type mice, but was absent in cardiac knockout (KO) mice lacking the cardiac GLUD1 gene encoding GDH. Moreover, in cardiac GDH KO mice, IPC neither activated the RISK or SAFE pathways nor reduced infarct size following 25 min of I/R as in wild-type mice. However, pharmacological pre-conditioning with hydrogen peroxide, bypassing the need for endogenously-generated signaling ROS by GDH, was equally effective at reducing infarct size in cardiac GDH KO mice as in wild-type mice. Cardioprotection by ischemic post-conditioning remained intact in cardiac GDH KO mice. Together, these findings identify mitochondrial matrix GDH as a critical redox enzyme required to activate cardioprotective signaling during IPC.

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Preconditioning in Neurological Applications

Adaptive Preconditioning in Neurological Diseases – Therapeutic Insights from Proteostatic Perturbations

Bertrand Mollereau, Laboratory of Biology and Modelling of the Cell, Ecole Normale Supérieure de Lyon, France

Ischemic Preconditioning: Mechanisms of Neuroprotection

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Dose-Dependent Neurocognitive Effects of Transcranial Infrared Laser Stimulation

Francisco Gonzalez-Lima, University of Texas at Austin, Departments of Psychology, Pharmacology and Toxicology, Austin, TX

Diet-Induced Metabolic Preconditioning of Brain Function and Plasticity through Epigenetics

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Exercise is Beneficial in Models of Retinal Disease

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Adaptive Preconditioning in Neurological Diseases – Therapeutic Insights from Proteostatic Perturbations

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In neurological disorders, both acute and chronic neural stress can disrupt cellular proteostasis, resulting in the generation of pathological protein. However in most cases, neurons adapt to these proteostatic perturbations by activating a range of cellular protective and repair responses, thus maintaining cell function. These interconnected adaptive mechanisms comprise a 'proteostasis network' and include the unfolded protein response, the ubiquitin proteasome system and autophagy. We have shown that unfolded protein response can be stimulated by preconditioning treatments, which confer resistance to a subsequent toxic challenge - the phenomenon known as ER-hormesis. I will discuss the impact of adaptive stress responses stimulated in diverse models of neurodegeneration.

Ischemic Preconditioning: Mechanisms of Neuroprotection

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In previous studies we demonstrated that both protein kinase C epsilon (PKCε) and resveratrol emulated ischemic preconditioning (IPC) in brain and that IPC, PKCε and resveratrol upregulate BDNF levels, which played a key role in neuroprotection. We also demonstrated that IPC and resveratrol preconditioning (RPC) induced neuroprotection via SIRT1 activation. The main goals of our studies are to define the specific molecular targets of IPC that promote ischemic tolerance and to further define the molecular mechanisms of a chronic ischemic tolerant state. In this presentation, I will review two major sites by which neuroprotection is achieved, namely mitochondria and synaptic terminals.

Dose-Dependent Neurocognitive Effects of Transcranial Infrared Laser Stimulation

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Transcranial infrared laser stimulation is a novel form of neuromodulation that has been successfully used to augment human cognitive functions such as attention, memory and executive functions. This laser stimulation uses low power (mW/cm^2) and high fluence (J/cm^2) laser at 1064 nm wavelength that has better penetration in deep tissues such as the brain. The photobiomodulation target of infrared light is the respiratory enzyme cytochrome oxidase, which is the terminal enzyme in the mitochondrial electron transport chain. Cytochrome oxidase is the major intracellular acceptor of infrared photons, which can be delivered non-invasively by transcranial infrared laser stimulation. Cytochrome oxidase is responsible for reduction of oxygen to water in the process of oxidative phosphorylation that produces metabolic energy in cells. Brain cells, in particular, are highly dependent on oxygen metabolism so that changes in cytochrome oxidase activity are closely related to neuronal function. However, little is known about the cognitive and neural dose-response effects of 1064 nm laser stimulation in humans. We investigated cognitive responses to repeated weekly sessions of laser stimulation in individuals 45-90 years old. The psychomotor vigilance test (sustained attention) and delayed match-to-sample test (working memory) were conducted immediately before the first laser treatment (Baseline: week 1), immediately after the first laser treatment (Acute: week 1), and on subsequent weeks after additional laser treatments (Chronic: weeks 2, 3, 4, 5). Reaction time (in msec) improved gradually over 3 weeks and then worsened gradually at weeks 4 and 5. A similar biphasic dose-response was found for the number of correct responses for the memory test. We also found that cerebral hemodynamic (oxygenation) and electrophysiological (EEG alpha power) dose-responses had peak effects at laser energy density doses of 70-97 J/cm^2 and decreased thereafter with higher doses. The results demonstrated that human neurocognitive effects of transcranial infrared laser followed hormetic dose-responses.

Diet-Induced Metabolic Preconditioning of Brain Function and Plasticity through Epigenetics

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Diet is one of the most crucial needs for species survival and adaptation, and overall health. We found that chronic fructose consumption disrupts crucial aspects of brain metabolism, and predisposes the brain to the effects of injury. Fructose disrupts hippocampal functional mitochondria bioenergetics, and aggravated the effects of brain injury on cell energy homeostasis, synaptic plasticity, and cognitive function [1]. In separate studies, we found that foods influence long-term brain plasticity by building an "epigenetic memory" that provides resistance to neurological challenges [2]. Early life exposure to the omega 3 fatty acid DHA promoted long-term protection against the detriments of adult exposure to a western diet using epigenetic mechanisms. Changes in DNA methylation is one of the most stable forms of epigenetic variability, and DHA-induced hypomethylation of *Bdnf* was associated to reduced BDNF transcription. The inherent properties of BDNF to act on plasticity and metabolism seem crucial to translate the effects of foods to the brain. Using a systems nutrigenomics approach, we found that fructose promotes selective transcriptomic and epigenomic alterations in the hypothalamus (central control of metabolism) and hippocampus (critical for cognitive functions) regions of the brain, engaging cell events related to metabolism, communication, inflammation, immune response, neuronal signaling, and cognition [3]. These molecular alterations converged with genes conferring genetic risks of metabolic and neuropsychiatric disorders in human genome-wide association studies. Remarkably, DHA supplementation counteracted the fructose-induced genomic reprogramming. The interplay between the effects of fructose and DHA consumption on brain pathogenesis highlights the impact of diet on determining resilience to neurological disorders. These studies are significant on the context of the health risk posed by the contemporary elevated fructose consumption on the current epidemic of metabolic and brain disorders.

1. Agrawal, R., et al., *Dietary fructose aggravates the pathobiology of traumatic brain injury by influencing energy homeostasis and plasticity.* J Cereb Blood Flow Metab, 2015.
2. Tyagi, E., et al., *Interactive actions of Bdnf methylation and cell metabolism for building neural resilience under the influence of diet.* Neurobiol Dis, 2015. **73**: p. 307-18.
3. Meng, Q., et al., *Systems Nutrigenomics Reveals Brain Gene Networks Linking Metabolic and Brain Disorders.* EBioMedicine, 2016. **7**: p. 157-66.

Exercise is Beneficial in Models of Retinal Disease

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Pre- and post-conditioning exercise benefits central and peripheral nervous system function in humans in healthy aging and in neurodegenerative disease. Similar effects along with structural correlates are seen in animal models. The effects of exercise are in part mediated by Brain-Derived Neurotrophic Factor (BDNF) in both humans and in animal models. We asked whether exercise might be directly beneficial to retinal and visual function. Retrospective studies hinted that increased activity levels are associated with delayed onset and diminished functional loss in age-related macular degeneration (AMD), but no studies, human or animal, prospectively addressed this rigorously. We therefore tested whether running on treadmills (involuntary exercise) or on wheels (voluntary exercise) protects against losses of retinal function and structure and losses of visual function in mouse models of AMD and retinitis pigmentosa (RP). Both involuntary and voluntary running partially but significantly maintained retinal function as measured by the electroretinogram (ERG) and visual acuity as measured by optokinetic tracking (OKT). Retinal morphology was also preserved, with fewer photoreceptor cells undergoing apoptosis and the retinal outer nuclear losing fewer nuclei. The beneficial effects were mediated in part by BDNF. BDNF levels in serum, hippocampus, and retina increased in exercised mice. Conversely, beneficial effects of exercise were prevented by co-treatment of mice with ANA-12, an antagonist of the BNDF receptor trkB. Increasing the duration or rate of treadmill running did not alter outcomes, nor did distance run on wheels correlate with outcomes, suggesting a threshold effect. We previously developed a 12-week spin cycling exercise regimen that significantly maintains cognitive function in aged human subjects. We currently are assessing effects of this regimen on visual function and biomarker levels in aged human subjects.

General Biomedical Implications of Preconditioning

Old Dog, New Tricks–Role of Hydrogen Sulfide and NAD^+ in Angiogenesis and Health

Abhirup Das, Alban Longchamp, James R. Mitchell, David A. Sinclair
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Does Energetic Stress Activate Mechanisms of Proteostatic Maintenance and Slowed Aging?

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Carbon Monoxide-Releasing Molecules (CORMs) as Novel Anti-Obesity Drugs.

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Stem *Cells-Based Therapy for Ischemic Stroke*

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Advancing Environmental Enrichment as a Pre-Clinical Model of Neurorehabilitation

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The Repeated Bout Effect in Exercise

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Old Dog, New Tricks–Role of Hydrogen Sulfide and NAD^+ in Angiogenesis and Health

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A progressive loss of capillary density and adequate blood flow is a major cause of human frailty and mortality. Understanding why it occurs and how to reverse it are key to future gains in human health. Here we present evidence that NAD^+-dependent SIRT1 expressed by endothelial cells is a key mediator of pro-angiogenic signal, vascular endothelial growth factor (VEGF), secreted from myocytes. Consistent with this, treatment of mice with NAD^+ precursor nicotinamide mononucleotide increases endurance by promoting SIRT1-dependent increase in capillary density in skeletal muscle during ischemia and aging, an effect that is further increased by co-treatment with H_2S donor sodium hydrosulfide or by exercise. We have also found that restriction of dietary sulfur amino acids in mice promoted VEGF expression and capillary growth in skeletal muscle, via eIF2alpha kinase GCN2 and the transcription factor ATF4. Activation this pathway also increased expression of cystathionine-gamma-lyase (CGL), an enzyme responsible for production of the pro-angiogenic gas, hydrogen sulfide (H_2S), in endothelial cells.

Taken together, our data suggest novel pathways by which amino acid deprivation and NAD^+ replenishment provide a powerful pro-angiogenic response. These findings have implications for peripheral artery disease and ischemic injury as well as for enhancing the response to exercise and treating a variety of age-related diseases caused by decreased blood flow.

Does Energetic Stress Activate Mechanisms of Proteostatic Maintenance and Slowed Aging?

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The clear health benefits of slowing the biological aging process led to formation of the trans-NIH Geroscience Interest Group to advocate for studies to “treat” the aging process. The scientific premise for developing treatments to slow aging is that aging is the major risk factor for most chronic diseases and, therefore, slowing aging simultaneously diminishes the risk of developing most chronic diseases. Many of the interventions shown to extend lifespan, healthspan, or both, involve energetic stress or activation of cell signaling associated with energetic stress. This presentation will focus on our studies that impose energetic constraints or activation of stress signaling with lifelong and transient calorie restriction, rapamycin treatment, thyroid/growth hormone disruption (i.e., Snell dwarf), Nrf2 activation, and exercise. These models provide evidence that proteostatic maintenance is a shared characteristic of slowed aging and may contribute to stress resistance and improved healthspan.

Carbon Monoxide-Releasing Molecules (CORMs) as Novel Anti-Obesity Drugs.

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Carbon monoxide (CO) is an endogenously produced gas derived from the metabolism of heme by heme oxygenase enzymes as well as from lipid peroxidation. Carbon monoxide-releasing molecules (CORMs) are a class of molecules which release CO in a variety of ways both *in vitro* and *in vivo*. We determined the effect of chronic low-level administration of a specific CO donor (CORM-A1) to prevent and reverse dietary-induced obesity as well as effects on metabolism and adipocyte biology in mice fed a high-fat diet. CORM-A1 (5 mg/kg) was administered via intraperitoneal injections over a 30-week period. Control mice received injections of an inactivated form which was devoid of CO (iCORM-A1). Chronic CORM-A1 treatment prevented as well as reversed established dietary-induced obesity with changes in body weight of ~30% as compared to iCORM-A1 and control mice. CORM-A1 treatment resulted in significant changes in body weight without any significant effect on food intake or activity as compared to iCORM-A1 and control mice. CORM-A1 treatment did result in significant increases in O_2 consumption which increased from 50-100% as compared to iCORM-A1 and control mice. Moreover, chronic CORM-A1 treatment also normalized high-fat diet induced hyperglycemia and hyperinsulinemia. Chronic CORM-A1 treatment also remodeled adipocytes to a “beige” phenotype with resulting increases in the levels of PGC-1□, NRF-1, and UCP-1. Chronic CORM-A1 treatment reduced the levels of high-fat diet induced HMGB1, a mediator of inflammation, in adipose tissue. Chronic CORM-A1 treatment was also able to reverse high-fat diet induced hepatic steatosis. In separate studies, we found that the beneficial effects of CORM-A1 on body weight and metabolism were not recapitulated by chronic inhalation of CO either at low (20 ppm) or high (200 ppm) levels. Our results demonstrate that CORMs are novel anti-obesity drugs and may also have benefit treating some of the associated pathologies related to obesity.

Stem *Cells-Based Therapy for Ischemic Stroke*

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Stroke remains a leading cause of disability in the world. Despite progress in understanding molecular mechanisms of neuronal cell death after stroke, widely effective treatment remains elusive. We have documented that endogenous neural stem cells (NSCs) can proliferate, migrate and differentiate into functional neurons to replace or repair damaged neurons after acute ischemic stroke. Conditional depletion of neurogenesis inhibits functional recovery after ischemic stroke either in young adult or aged animals. Yet, patients who survive an acute stroke are typically left with fixed anatomical damage, which eventually transforms a brain cavity and results in permanent neurological deficits. Therefore, NSCs may not be able to reconstitute the lost neural tissue and restore the functional circuitry at chronic stage of stroke due to the brain cavity. To help elucidate the potential of cell replacement therapy in stroke, we found that transplantation of human ESC-derived NSCs with Matrigel scaffolding resulted in improved histologic and behavioral outcome in animal model of stroke. However, many issues remain to be addressed before clinical application of this strategy becomes feasible, as Matrigel is a gelatinous protein mixture extracted from EHS mouse sarcoma cells. To address this issue, we generated gel-like scaffold from serum with ideal properties, and treated patients with ischemic stroke using autogenous stem cells and serum-derived scaffold. We found that the motor deficits and tissue damage post-stroke were significantly improved after transplantation, suggesting that stem cells-based tissue engineering may be a clinically effective therapeutic strategy for repairing the damaged brain tissue in the chronic phase after stroke.

Advancing Environmental Enrichment as a Pre-Clinical Model of Neurorehabilitation

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Traumatic brain injury (TBI) is a significant health care issue that affects approximately ten million individuals worldwide each year. Although motor deficits are common, cognitive impairments are often more pronounced and prolonged, which adversely affects quality of life. Given the devastating consequences of TBI, the development and initiation of treatment paradigms capable of promoting neurobehavioral and cognitive recovery is vital. One approach that has steadily gained momentum as a potentially advantageous therapy for experimental TBI is environmental enrichment (EE). EE consists of providing rats a milieu that is conducive for engaging in sensory stimulation and physical exercise in an expansive social environment that may be akin to multi-modal clinical rehabilitation. The typical EE paradigm, which consists of presenting enrichment immediately and continuously after TBI, has consistently demonstrated significant improvement in motor function, spatial learning, and memory retention. Furthermore, EE induces significant histological protection as demonstrated by smaller cortical lesions and decreased hippocampal $CA_{1/3}$ cell loss after TBI. Importantly, the benefits of EE are observed across several models of brain trauma. However, to further advance EE as a relevant preclinical model of neurorehabilitation studies have been conducted that delay the onset of EE and also abbreviate the amount provided. These studies are designed to mimic the clinic where TBI patients will not engage in rehabilitation until after critical care has run its course. Moreover, once in rehabilitation, the amount provided is not continuous. The data show that EE is effective even when delayed and abbreviated, which strengthens its validity as a preclinical model of neurorehabilitation. Current studies are evaluating the effects of EE provided prior to TBI to determine the effects of preconditioning on brain trauma and functional recovery.

The Repeated Bout Effect in Exercise

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Low levels of stress improve the capacity of cells and organisms to withstand greater stress, which is referred to as hormesis, is often seen in exercise. During exercise, the body is exposed to thermal, metabolic, hypoxic, oxidative and mechanical stress, which are important for our body to adapt to exercise. Exercise consisting of lengthening (eccentric) muscle contractions induces muscle damage that is indicated by prolonged decreases in force generating ability, delayed onset muscle soreness (DOMS), and increased levels of muscle proteins such as creatine kinase (CK) and myoglobin (Mb) in the blood. However, skeletal muscles adapt to exercise-induced damage rapidly after the initial exposure, and the magnitude of muscle damage induced by subsequent bouts of the same exercise is significantly reduced. This is represented by faster recovery of force generating ability, attenuated DOMS, and smaller increases in CK and Mb. This adaptation is referred to as the repeated bout effect (RBE). The protective characteristics of the RBE are conferred by “non-damaging” exercises such as low-intensity eccentric contractions and maximal isometric contractions at a long muscle length. The protective effect is also endowed upon the contralateral (non-exercised) limb. Our recent study showed that changes in indirect muscle damage markers were smaller after the second bout than those after the first bout, when the opposite arm performed the second exercise bout at 24 hours, 7 days and 28 days, but not at 0.5, 6 and 12 hours and 56 days. Although the underpinning mechanisms of the RBE are not fully understood, recent studies have provided insight on how neural adaptations, alterations to muscle mechanical properties, structural remodeling of the extracellular matrix, inflammation, and biochemical signaling contribute to the emergence of the RBE. In the presentation, several aspects of the RBE are introduced, and its mechanisms in relation to hormesis will be discussed.

Environmental Implications of Preconditioning

The Role of Excitation Events in Low Dose Radiobiology
Carmel Mothersill and Colin Seymour, Department of Medical Physics and Applied Radiation Sciences, McMaster University, Hamilton, Ontario, CANADA

Is Ionizing Radiation Harmful at any Exposure?
Edouard I. Azzam, Rutgers, New Jersey Medical School, Newark, NJ

Low-Dose Dose-Response for *In Vitro* Nrf2-ARE Activation in Human Liver HepG2 Cells
Kenneth T. Bogen, Exponent Health Sciences, Oakland, CA

The Role of Excitation Events in Low Dose Radiobiology

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Irradiation of biological material is well known to cause both ionization and excitation events. Ionization involves complete ejection of an electron from an atom resulting in multiple secondary ionizations leading to free radical formation. Free radical interactions with DNA are considered to be the main way that ionizing radiation leads to mutations in DNA although direct “hits” by the ejected electrons along the track can also cause DNA damage. Much less attention is given to the role of excitation in mediating biological effects of ionizing radiation. The main mechanisms by which non-ionizing electromagnetic radiations such as UV, “blue light”, ELF-EMF etc are thought to cause biological effects are through excitation decay where biophotons are emitted by the biological molecules which are excited by the energy deposition. These biophotons are in turn absorbed by other molecules depending on their wavelength. This presentation will review the field of biophoton radiobiology and will discuss recent data from our laboratory which has shown that secondary UV and other EM emissions are emitted from cells and organisms exposed to low doses of ionizing radiation. These secondary emissions appear to trigger novel signalling events and to be involved in so-called “non-targeted” effects such as bystander effects and induction of genomic instability. Finally the possible role of such processes in radiation protection will be discussed.

Is Ionizing Radiation Harmful at any Exposure?

Edouard I. Azzam, Rutgers New Jersey Medical School, Newark, NJ

The health risks to humans and non-human biota exposed to low dose ionizing radiation remain ambiguous and are the subject of intense debate. The need to establish risk assessment standards based on the mechanisms underlying low-level radiation exposure has been recognized by regulatory agencies as critical to adequately protect people and to make the most effective use of our national resources. Here, we briefly review laboratory-based evidence showing that the molecular and biochemical changes induced by low doses of radiation differ from those induced by high doses. In particular, an array of redundant and inter-related mechanisms act in both prokaryotes and eukaryotes to restore DNA integrity following exposures to relatively low doses of sparsely ionizing radiation. Furthermore, the radiation-induced protective mechanisms often overcompensate and minimize the mutagenic potential of the byproducts of normal oxidative metabolism. In contrast to adaptive protection observed at low doses of sparsely ionizing radiation, there is evidence that even a single nuclear traversal by a densely ionizing particle track can trigger harmful effects that spread beyond the traversed cell and induce harmful effects in the nearby bystander cells. *In vivo* studies examining whether exposure to low dose radiation at younger age modulates the latency of expression of age-related diseases such as cancer, together with studies on the role of genetic susceptibility, will further illuminate the magnitude of risk of exposure to low dose ionizing radiation.

Low-Dose Dose-Response for *In Vitro* Nrf2-ARE Activation in Human Liver HepG2 Cells

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Kelch ECH associating protein 1 (Keap1), nuclear factor erythroid 2 (NFE2)-related factor 2 (Nrf2), and the antioxidant response element (ARE) form components of a master regulatory signaling pathway that coordinates redox homeostasis with a wide variety of direct cytoprotective responses to endogenous and environmental stressors, and additionally mediates stem-cell state shifts between quiescence/maintenance, active proliferation, and differentiation. Detailed dose-response data were recently reported for *in vitro* Nrf2-ARE activation in human liver HepG2 cells containing either a ARE-*bla* or ARE-*luc* reporter at 12 different concentrations of each of 15 chemicals (Shukla et al., Environ Health Perspect 2012; 120(8):1150-6). A more detailed dose-response (DR) assessment of those data was performed using corresponding raw study data (kindly provided the study authors) that were combined over all chemicals that clearly exhibited a positive response at ≥1 concentration (yielding n = 531 and 179 DR data for 9 and 7 chemicals using the ARE-*bla* and ARE-*luc* assays, respectively). Three-parameter linear/k^{th}-degree polynomial fits obtained by inverse-variance-weighted nonlinear regressions to each combined set of (ARE-*bla*- or ARE-*luc*-assay) ARE activation data provide good overall fits to those data (R^2 = 0.99 or 0.91, $p_{fit} > 0.99$) and each incorporate a highly significantly negative initial linear slope ($p = 4 \times 10^{-5}$ or 0.00025) and an overall J-shaped DR pattern. Results from this first analysis of high-resolution ARE-response data are consistent with previous theoretically based predictions and with non-monotonic DR patterns generally considered to characterize ARE-mediated cellular responses to different levels of oxidative stress. These results also imply that if sufficiently sustained ARE activation by reactive (including genotoxic) chemicals can increase tumor risks simply by increasing the number of stem cells recruited into epigenetically programmed states of adaptive hyperplasia, low-dose DR patterns for such increased risks are likely to be nonlinear.

Posters

Gene Expression as a Radiation Dosimeter in *Drosophila melanogaster*

Michael P. Antosh and Samana Shrestha, University of Rhode Island, Physics Department, University of Rhode Island, Kingston, RI
Adam Vanasse, University of Rhode Island, Kingston, RI
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Impact of Chronic Low-dose Tritium Radiation Exposure on Lung Carcinogenesis in Laboratory Mice

Laura Bannister and Mandy Serran, Canada Nuclear Laboratories, Chalk River, ON, Canada

The Importance of Hormesis and the Effects of UVA Irradiation on *Drosophila melanogaster* Performance

Raymond Berry III and Dr. Giancarlo Lopez-Martinez, New Mexico State University, Biology, Las Cruces, NM

Evidence that Lifelong Low Dose-Rates of Ionizing Radiation Increase Lifespan in Long- and Short-Lived Dogs

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Treatment of Alzheimer Disease with CT Scans: Update on a Case Report

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Anoxia Preconditioning as a Hormetic Treatment in *Tenebrio molitor*

Alyssa De La Torre and Dr. Giancarlo Lopez-Martinez, New Mexico State University, Biology, Las Cruces, NM

Radiotherapy for Pertussis: An Historical Assessment

Gaurav Dhawan and Edward J. Calabrese PhD, Environmental Health Sciences, University of Massachusetts, Amherst, MA 01003
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Metabolic Syndromes Among Diabetic Pre- And Postmenopausal Women

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Irradiation Hormesis Shows Activity Improvement in a *Drosophila melanogaster* Parkinson's Disease Model

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Low-dose Radiation Exposure in Early Life Stimulates Reproductive Performance in a Short-lived Model of Aging

Alexander Shephard, Vadim Aksenov, and Jonathan Tran, McMaster University, Biology, Hamilton, ON, Canada
C. David Rollo, McMaster University, Biology, Hamilton, ON, Canada

***In-Utero* Low Dose Irradiation Effects on Post-Natal Growth and Blood Pressure in C57Bl Mice**

Shayen Sreetharan, McMaster University, Department of Biology, Hamilton, Ontario, Canada
Lisa Stoa, McMaster University, Department of Medical Physics and Applied Radiation Sciences, Hamilton, Ontario, Canada
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Acute Low Dose Ionizing Radiation Stimulates the Innate Immune System of the Cricket (*Acheta domesticus*): Evidence for Hormesis

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Gene Expression as a Radiation Dosimeter in *Drosophila melanogaster*

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Biological indicators would be of use in radiation dosimetry in situations where an exposed person is not wearing a dosimeter, or when physical dosimeters are insufficient to estimate the risk caused by the radiation exposure. In this work, we investigate the use of gene expression as a dosimeter. Gene expression analysis was done on 15222 genes of *Drosophila melanogaster* (fruit flies) at days 2, 10 and 20 post irradiation, with x-ray exposures of 10, 1000, 5000, 10000 and 20000 Roentgens. Several genes were identified which could serve as a bio dosimeter in an irradiated drosophila melanogaster model. Many of these genes have human homologs. 7 genes showed a linear response (R^2 > 0.9) with dose at all time points. One of these genes, Irbp, is a known DNA repair gene and has a human homolog (XRCC6).

The lowest dose, 10 R, is very low for fruit flies. If the lowest dose is excluded, 18 genes showed a linear response with dose at all time points. This includes all 7 genes that were linear with all radiation doses included. Of these 18 genes, 6 have human homologs and 11 have known functions.

The expression of this panel of genes, particularly those with human homologs, could potentially be used as the biological indicator of radiation exposure in dosimetry applications.

Impact of Chronic Low-dose Tritium Radiation Exposure on Lung Carcinogenesis in Laboratory Mice

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Lung cancer is the leading cause of cancer-related deaths death in the USA and worldwide. Mouse models of primary lung carcinogenesis represent a very valuable tool for the study of tumor initiation, promotion, and therapy and for establishing experimental linkage between etiological risk factors and lung cancer. Strain A (A/J) mice have a high incidence of spontaneous lung tumor development and are susceptible to carcinogen-induced lung tumors. The susceptibility of A/J mice to develop lung tumors is related to genetic poylmorphisms in the *Pulmonary adenomasensitivity* (*Pas*) locus and a propensity of A/J mice to incur activating mutations in the *K-ras* proto-oncogene following treatment with carcinogens. Previous results from other laboratories have shown that fractionated low-dose gamma radiation exposure inhibited the development of chemically-induced lung adenomas in A/J mice. The objective of our current study is to examine the risks of lung cancer initiation and progression and underlying cellular and molecular mechanisms following low-dose chronic tritium (beta radiation) exposures of A/J mice. Lung cancer risk will be monitored in A/J mice injected with bezo(a)pyrene (BaP, a known toxicological component of cigarette smoke) and subsequently exposed to low-dose tritium radiation in the form of tritiated drinking water for two months duration. In parallel with tumor incidence and latency measurements in BaP and tritium-exposed mice, mechanistic processes through which cancer risk may be altered will be examined to understand the impact of low-dose radiation exposure on immune function and cellular and molecular pathways of carcinogenesis.

The Importance of Hormesis and the Effects of UVA Irradiation on *Drosophila melanogaster* Performance

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Increasing evidence suggests that ultraviolet (UV) radiation is one of the most ubiquitous environmental hazards that impact every living creature. Environmental stress, like UV radiation, leads to the production of reactive oxygen species (ROS) which are the damaging force behind environmental insult by attacking lipids, proteins, and DNA. It is this type of cellular damage that has been linked to multiple conditions in humans ranging increased risk of infectious disease to cancer. When the response to environmental stress is biphasic resulting in low level stress being protective while high levels of the same stressor being detrimental, we termed this hormesis. Low levels of ROS are not harmful to cells and play an important role in cell signaling and the induction of endogenous defense pathways. The protective effects of hormesis in animal models includes improved treatment survival, and mating, extended longevity, and improved performance at old age (i.e. mating); amongst others. Here, we test whether mild exposure to UVA is hormetic and helps lessen damage at the cellular level in *Drosophila melanogaster*. By using the vinegar fly, *Drosophila melanogaster*, as our model organism, we can add to a well understood genetic model with a vast array of genetic tools that are available. This may help define a strong hormetic response, and will allow for more precise evaluation of genetic and protein targets of hormetic treatment. Flies were exposed to 0, 30, or 60 minutes of ultraviolet radiation A (nm = 365) at a rate of mW/m^2. Different rates of UVA exposure, produced by the distance from the source, show differences in treatment survival/adult emergence and flight ability. We suggest that UVA has the potential to have a beneficial hormetic affect at low doses that increase performance.

Evidence that Lifelong Low Dose-Rates of Ionizing Radiation Increase Lifespan in Long- and Short-Lived Dogs

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After the 1956 radiation scare to stop weapons testing, studies focused on cancer induction by low-level radiation. Concern has shifted to protecting “radiation-sensitive individuals.” Since longevity is a measure of health impact, this analysis reexamined data to compare the effect of dose-rate on the lifespans of short-lived (5% and 10% mortality) dogs and on the lifespans of dogs at 50% mortality. The data came from two large-scale studies. One exposed 10 groups to different gamma dose-rates; the other, 8 groups to different lung burdens of plutonium. Reexamination indicated that normalized lifespans increased more for short-lived dogs than for average dogs, when radiation was moderately above background. This was apparent by interpolating between the lifespans of non-irradiated dogs and exposed dogs. Optimum lifespan increase appeared at 50 mGy/year. The threshold for harm (decreased lifespan) was 700 mGy/year for 50% mortality dogs and 1100 mGy/year for short-lived dogs. For inhaled alpha-emitting particulates, longevity was remarkably increased for short-lived dogs below the threshold for harm. Short-lived dogs seem more radiosensitive than average dogs and they benefit more from low radiation. If dogs model humans, this evidence would support a change to radiation protection policy. Maintaining exposures “as low as reasonably achievable” appears questionable.

Treatment of Alzheimer Disease with CT Scans: Update on a Case Report

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Alzheimer disease (AD) primarily affects older adults. This neurodegenerative disorder is the most common cause of dementia and is a leading source of their morbidity and mortality. Patient care costs in the United States are about 200 billion dollars and will more than double by 2040. The case report describes the remarkable improvement in a patient with advanced AD in hospice who received 5 computed tomography (CT) scans of the brain, about 40 mGy each, over a period of 3 months. The mechanism appears to be radiation-induced up-regulation of the patient's adaptive protection systems against AD, which partially restored cognition, memory, speech, movement, and appetite. In February 2016, recognizing that the improvement following the treatment with 5 CT scans would likely be transitory, the patient's spouse requested booster scans every 4 to 5 months. The interval between these treatments was shortened following the observation of a slight decline in the patient's condition. Meanwhile, the patient's spouse has been receiving CT scan treatments to alleviate symptoms of Parkinson disease.

Anoxia Preconditioning as a Hormetic Treatment in *Tenebrio molitor*

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All organisms share the common struggle of maintaining homeostasis despite repeated exposures to environmental stressors throughout their lifetime. Most organisms are constantly exposed to oxidative stress endogenously through multiple exogenous sources, simultaneously combined with other stressors. Oxidative damage results from the accumulation of reactive oxygen species (ROS) fueled by environmental stress, which causes lasting effects that are potentially detrimental to organismal performance and health. However, when exposed to low levels of a stressor, which is lethal at high doses, a biphasic response known as hormesis protects against cellular damage and diminished performance.

Physiological oxygen conditioning is known to elicit beneficial effects across different animal models including humans. By lowering oxygen concentration in the cell, mitochondria enter a state of defense in preparation for resulting ROS production through the upregulation of protective genes such as antioxidants and molecular chaperones. These defensive products are then readily available when excessive free radical damage occurs.

For every biological problem, there is an organism for which it can be most conveniently studied. Although *Tenebrio molitor*, known as the mealworm beetle, is classified as a non-model organism, it is most useful in proof of concept studies. Here we use *Tenebrio molitor* as a model to investigate the hormetic effects of oxygen conditioning on overall organismal performance. Male and female mealworm beetles were exposed to anoxic conditions (0% oxygen) for different time periods during development. Longevity, fecundity, activity, and various biochemical markers were measured in order to determine the extent of the hormetic effects of anoxia during development. Exposure to anoxic conditions leads to a delay in developmental emergence. The results indicate that anoxic hormesis is dependent on dose, gender, and stage of life.

Radiotherapy for Pertussis: An Historical Assessment

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X-ray therapy was used to treat pertussis/whooping cough during a thirteen-year period from 1923-1936 in North America and Europe. Twenty studies from clinicians in the U.S reported that approximately 1500 cases of pertussis were treated by X-ray therapy usually with less than 0.5 Erythema Dose (ED). Young children (< 3 years) comprised about 70-80% of the cases, with the age of cases ranging from as young as one month to years. In general, symptoms of severe coughing, vomiting episodes and spasms were significantly relieved in about 85% of cases following up to three treatments, while about 15% of the cases showed nearly full relief after only one treatment. The X-ray therapy was also associated with a marked reduction of mortality of young (< 3 years) children by over 90%. Despite such reported clinical success from a wide range of experienced researchers, use of X-rays for the treatment of pertussis in young children was controversial, principally due to concerns of exposure to the thymus and thyroid even with the availability of lead shielding. By the mid-1930s, the treatment of pertussis cases via vaccine therapy came to dominate the therapeutic arena and the brief era of a radiotherapy option for the treatment of pertussis ended.

Key Words: pertussis, whooping cough, x-rays, radiotherapy, history of science, hormesis

Metabolic Syndromes Among Diabetic Pre- And Postmenopausal Women

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The risk of having metabolic syndrome is closely linked to overweight and obesity and a lack of physical activity. It increases the chances of type 2 diabetes. The situation gets worsened in postmenopausal women. For the present cross-sectional study 595 women were recruited. 330 were premenopausal and 265 were postmenopausal. The anthropometric measurements like height, weight, waist circumference and skin folds like biceps, triceps, supra iliac and sub scapular were taken on each subject using standard methodology. Blood pressure was measured with mercury sphygmomanometer. Body mass index, waist hip ratio, weight height ratio, body fat, percent body fat was calculated. Fasting blood samples were taken for biochemical analysis. A person was considered to be having diabetes if she was already diagnosed case of diabetes and /or was on treatment or current fasting blood glucose was ≥126 mg/dl.

Lipid profile of diabetic subjects was assessed. Total serum Cholesterol (TC), triglycerides (TG) and lipoproteins; heavy density lipoproteins (HDL-C) and low density lipoproteins (LDL-C) were estimated. Statistically significant differences were observed in the mean values of most of the anthropometric and physiological variables between normal and diabetic pre- and postmenopausal women. Values were on higher side in postmenopausal women as compared to their premenopausal counterparts although statistically higher values were observed in case of biceps, percent body fat, fat mass and pulse pressure only. Similar was the status of lipid profile. Statistically higher values of TC, TG, LDL-C and lower value of HDL-C was observed among diabetic postmenopausal women as compared to premenopausal women. Lower levels of estradiol and higher age among postmenopausal women could be among the various risk factors for the occurrence of diabetes. Balanced diet and regular exercise is recommended for a healthy life style.

Presenting Author: Kawaljit Kaur

Irradiation hormesis Shows Activity Improvement in a *Drosophila melanogaster* Parkinson's Disease Model

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A screen of expressed transcripts in response to anoxia-conditioning hormesis challenged by x-ray irradiation revealed a number of protective gene pathways in common between anoxia and irradiation. This led us to explore the potential of low level irradiation at eliciting a hormetic response. Low level irradiation would still lead to the production of reactive oxygen species (ROS), but a lower irradiation dose (0.3% of previous dose) ROS would be produced at a much lower rate. This small amount of ROS is most likely benign but ample enough to trigger cellular defenses and immune responses, without leading to substantial oxidative damage. Here we test whether low dose x-ray irradiation can elicit a hormetic cellular protective response. Previous studies reveal that doses between 250 and 500 cGy were not harmful to flies and did not lead to declines in emergence, survival, or flight ability. In fact some of these metrics were improved. These doses were chosen because they are in the range of doses received during therapy treatments or imagining studies. Inspired by this preliminary data, we decided to test the effects of irradiation hormesis in a line of *Drosophila* flies (PD) that had been transgenically modified to show signs of Parkinson's disease (i.e. skeletal motor dysfunction and diminished coordination). Radiation-treated PD flies were able to regain mobility and therefore performed at a higher level than untreated PD. Our data suggests that low levels of x-ray irradiation might be a promising treatment for translational research that can someday provide relief and hope to Parkinson's disease patients.

Low-dose Radiation Exposure in Early Life Stimulates Reproductive Performance in a Short-lived Model of Aging

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Reproduction is one of the most stressful and energetically demanding periods of an organism's lifetime, and individuals must typically balance investment in reproductive processes against their own survival. The hormesis paradigm predicts that exposure to mild stressors in early life might prime the individual to better withstand stressful events later in life, thereby improving health and longevity. However, whether early-life exposure to mild stress can simultaneously improve reproductive performance and longevity has not been specifically addressed. To test this question, we employed the cricket (*Acheta domesticus*), an established short-lived model of aging and free radical biology. Juvenile crickets (aged 14 d) were exposed to an acute dose of 137Cs gamma radiation (1 Gy, 2 Gy, 4 Gy, or 5.5 Gy; dose rate = 0.25 Gy/min) and then assessed for several measures of reproductive performance in adulthood (age 55 – 65 days). Crickets exposed to relatively low radiation doses (1 Gy and 2 Gy) had a higher adult reproductive output and laid larger eggs compared to controls, but there were no effects on their longevity or the quality of their offspring (measured by egg hatching success and starvation resistance of the hatchlings). Crickets in the 4 Gy treatment experienced a slightly lower reproductive output, but egg size, lifespan, and offspring quality were not negatively affected. In the 5.5 Gy treatment, reproductive output, lifespan, and offspring quality were all negatively impacted. This research supports the idea that early-life radiation hormesis can improve some aspects of adult reproductive performance without significant costs for longevity. Future work should further explore the potential health and fitness benefits of early life hormesis.

In-Utero Low Dose Irradiation Effects on Post-Natal Growth and Blood Pressure in C57Bl Mice

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Ionizing radiation exposure during pregnancy presents a two-fold problem: concern for the mother and the unborn child. Fetal exposures to ionizing radiation (the majority at very low doses) largely occur through diagnostic radiography or occupational exposures of the pregnant mother. The “fetal programming” phenomenon, which involves permanent changes in offspring phenotype due to a stress experienced *in-utero* has been demonstrated in cardiometabolic disorders such as hypertension. Fetal programming has been reported to involve a mechanism of oxidative stress, and therefore the potential role of prenatal ionizing radiation exposure on offspring cardiovascular health and disease following birth is of relevance, even at low doses.

Pregnant wildtype C57Bl/6J mice were irradiated on gestational day 15 with whole-body ^{137}Cs gamma radiation at nominal doses of 5, 10, 50, 100, 300 or 1000 mGy. Mothers were allowed to deliver, and offspring were followed. Post-natal measurements of weight and blood pressure (mean arterial pressure, measured using tail-cuff plethysmography) were completed weekly until 16 weeks of age.

Significant reduction in body weight was observed in female pups consistently over the course of post-natal development at higher doses (100, 300, and 1000 mGy) compared to sham-irradiated female pups, with the greatest reduction in weight at 1000 mGy. A significant increase in mean arterial pressure was also observed at 8 weeks of age in female pups at the same higher doses. Consistent, significant increase in male pup weights was observed over the course of post-natal development at lower doses (5, 10, and 50 mGy) relative to sham-irradiated male pups. Further investigation into the effects of ionizing radiation exposure during pregnancy (particularly at low doses) is important due to preliminary evidence of programming effects with prenatal exposure to higher doses of ionizing radiation.

Acute Low Dose Ionizing Radiation Stimulates the Innate Immune System of the Cricket (*Acheta domesticus*): Evidence for Hormesis

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The direct damage from high doses of ionizing radiation or the ultimate generation of reactive oxygen species (ROS) can contribute to accumulating cellular damage. The immune system, an important regulator for health and survival, is extremely radiosensitive in humans. Studies on low dose ionizing radiation, however, have shown hormetic effects with the innate immune system in mammals. Insects have an immune system similar in function to the innate immune system of vertebrates. Investigations of effects of low dose radiation on insect immunity have been largely unexplored. We examined impacts of 137Cs gamma radiation (0.2, 0.5, 0.75, 1, 5, and 15 Gy; dose rate = 0.25 Gy/min) on measures of innate immunity in adult male crickets (*Acheta domesticus*). Crickets exposed to high doses (above 5 Gy) experienced negative impacts on hemocyte concentration, phenoloxidase activity and encapsulation ability. Lower doses (0.5 and 0.75 Gy) induced a ~60% increase in hemocyte concentration compared to controls, which persisted up to two-weeks post irradiation. Total phenoloxidase activity followed similar trends. Improved encapsulation ability was also observed at 0.5 and 0.75 Gy. This data provides promising evidence in support of the hormesis paradigm. Benefits could extend to an improved ability to mount an immune response and protection against infection in insects exposed to low dose radiation.