The 14th Annual International Dose-Response Conference

PRECONDITIONING: IN BIOLOGY AND MEDICINE

MECHANISMS AND TRANSLATIONAL RESEARCH

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ABSTRACT BOOK

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Preconditioning by Ethanol Ingestion Prevents Postischemic Microvascular and Tissue Dysfunction: Role of the Immune System

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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 immune cells 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 coactivation of xanthine oxidase and NADPH oxidase-dependent oxidant production, adenosine A2-receptors, and endothelial nitric oxide synthase. Reactive oxygen species and nitric oxide interact to elicit transient-receptor potential vanilloid-1 (TRPV1) channel-dependent release of calcitonin gene-related peptide (CGRP) from capsaicin-sensitive sensory neurons. In turn, CGRP stimulates dendritic cells and/or CD8+ T cells to produce tumor necrosis factor alpha (TNFα). CGRP- and TNFα-dependent activation of tissue resident neutrophils elicits the release and activation of matrix metalloproteinase-9 (and perhaps other proteases) that liberate matricryptins from the extracellular matrix. These proteolytic digestion products activate large conductance, calcium-activated potassium 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 a grant from the National Institutes of Health (AA014945).

Hydrogen Sulfide: A Mediator and Modulator of Conditioning

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Recent studies have shown that exogenous hydrogen sulfide (H_2S), or H_2S-donating compounds, can be used to pre-, per-, post- or remote condition tissues and organs thereby protecting them from ischemia/reperfusion injury (I/R). Endogenous H_2S may also be a key couple in hypoxic conditioning.

Constitutive cysteine metabolism accounts for most H_2S production in tissues. Under normoxic conditions H_2S is rapidly oxidized in the mitochondria and intracellular concentrations are minimal. During hypoxia, H_2S oxidation fails and the rise in intracellular H_2S initiates downstream effector responses consistent with hypoxic conditioning. Exogenous H_2S activates similar effectors. Much H_2S signaling occurs through interaction with protein cysteine sulfur (R-SH). H_2S can modify other signals that act at this R-SH or H_2S can directly affect enzyme activity or protein structure. H_2S can reverse the effects of sulfenyl, S-nitrosothiol and S-guanylated proteins, reduce disulfide bonds and interact with vicinal and solitary R-SH. Numerous enzymes and ion channels have been shown to be activated/inactivated by H_2S and H_2S can signal at the nuclear level to regulate various pro- or anti-survival strategies. Many of these are consistent with conditioning responses. H_2S may also react directly with nitric oxide (NO) to form nitroxyl (HNO) and mediate additional signaling pathways. Sulfide salts traditionally used to generate H_2S are being replaced by slow-release compounds allowing tighter control of H_2S delivery. These compounds can be bound to other drugs, most notably non-steriodal anti-inflammatory drugs (NSAIDs), to augment therapeutic effects. Especially notable are “NOSH” NSAIDs that release both NO and H_2S. Recent developments allowing intracellular targeting of H_2S delivery offer more specific control of H_2S actions and suggest a bright future for H_2S therapies. Support: NSF IOS-1051627, IOS-144630.

Adaptive Response: Modulation of Response by Low Dose Diagnostic Radiation, Exercise, and Diet

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The adaptive response induced by a variety of stresses has been studied in many cell systems and is a highly conserved protective mechanism. We have investigated the adaptive response in mice using low doses of diagnostic radiation, oxidative metabolic stress from exercise, or a complex dietary supplement. We have shown that these three types of exposures have similar and important impacts on modulating risk. Multiple exposures to low dose CT quality X-rays modified mechanisms associated with a prior high cancer-inducing exposure and significantly increased lifespan. CT scans and exercise induced similar protective responses and were not additive, indicating a common mechanism. A complex dietary supplement was also effective at inducing significant radiation resistance in mice. Mice consuming the diet lived longer, had less overall cancer, and had fewer metastases. Overall, the mechanisms associated with these adaptive responses show that radiation exposure does not follow a linear non-threshold model for risk.

Preconditioning, Nanoneuropharmacology and the BRAIN Initiative: Neuroethical Obligations and Responsibilities

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The use of very low doses of ligands has gained considerable attention in light of the viability and value of such techniques to facilitate entry and action at specific targets in the central nervous system. The delivery of nanogram concentrations of various molecules may exert activity in both neuronal and glial tissues via engagement of receptor- and ionophore-linked, and/or non-receptor-linked (e.g. -membrane-based allosteric modulatory) mechanisms. These primary actions are capable of inducing processes that amplify and integrate several stimulatory and/or inhibitory signals that exert short-, intermediate-, and long-term effects ("downstream") within target tissues, cells and the networks with which they interact. In this light, further elucidation and understanding of very low dose (viz.-nanoneuropharmacological) mechanisms, actions and effects may provide opportunities to study and develop neurotropic agents with optimized, site- and pathway-specific effects, and relatively low (adverse or undesirable) side effects.

However, some caution is warranted. While (neuro)biological systems respond to very low concentrations of various ligands, the direct attribution of large-scale or whole-systems effects to such local mechanisms may be exaggerative, if not erroneous, as reflective of both a scaling error and fallacious assertion). The United States' *Brain Research through Advancing Innovative Neurotechnologies* (*BRAIN) Initiative* is positioned to prompt and support studies that employ new, and novel applications of neuroscientific techniques and tools to assess and affect the structure and function of neurological systems operative in cognition, emotion and behavior, as viable targets for therapeutics and for improving health and various aspects of human performance. Apropos this agenda, it is herein posited that important goals, tasks and opportunities reside in accurately understanding, and defining realistic application(s) of nanopharmacological responses and effects in neurological tissues and systems in ways that enable utility in clinical neurology, psychiatry, and physiatry.

The relative nascence – and novelty – of these approaches foster both considerable expectation (as regards positive therapeutic benefits) and apprehension (relevant to possible unanticipated effects), which have become focal to neuroethical consideration and engagement. Yet, the possibility of risk and burden need not – and arguably should not – preclude or proscribe continued research in this field. To the contrary, it is argued that (1) there is a definable and defensible neuroethical obligation for continuing research in nanoneuropharmacology, as well as (2) an equally robust necessity to undertake such investigation – and the translation of its outcomes and products – with responsibility to address neuroethical issues and questions, and mitigate and resolve various problems in order to sustain the field and methods of nanoneuropharmacology as a scientific and social good.

NEUROLOGICAL SESSION

Significance and Mechanisms of Ischemic Postconditioning against Stroke

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Unleashing the Brain's Endogenous Neuroprotective Strategies through Toll-Like Receptors

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Dietary Preconditioning Limits Neurological Impairment in Stroke Models

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Microglia Regulate Blood Clearance in Subarachnoid Hemorrhage by Heme Oxygenase-1

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Significance and Mechanisms of Ischemic Postconditioning against Stroke

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Stroke remains one of the leading causes of human death and disability worldwide, yet treatments are very limited. Therefore, it is necessary to explore novel neuroprotectants for stroke therapy. One of novel concepts is ischemic postconditioning (IPostC), which is defined in contrast with ischemic preconditioning (IPreC). While IPreC refers to a sub-lethal, brief ischemia before stroke onset, IPostC refers to a mechanical interruption of reperfusion, as if IPostC is conducted when the stimulus of IPreC is removed before stroke onset to after reperfusion. Both IPreC and IPostC protect against brain injury. Nevertheless, as the occurrence of most strokes cannot be predicted, IPostC is more clinically relevant. Now, the concept of IPostC has been expanded from reperfusion interruption to a broad range of intervention, including hypoxic postconditioning, remote postconditioning, and pharmacological postconditioning. The aim of this presentation is to review the progress of IPostC in stroke research. I will summarize the IPostC models, therapeutic time windows, and the underlying protective mechanisms. Both rapid and delayed IPostC will be discussed; the protective mechanisms of IPostC include the involvement of reactive oxygen species (ROS), apoptosis, inflammation, and neuronal survival signaling pathways, such as the Akt/mTOR pathways. These protective mechanisms will be compared with those of IPreC. Lastly, future research directions of IPostC will be discussed.

Keywords: ischemic postconditioning, stroke, Akt, mTOR

Unleashing the Brain's Endogenous Neuroprotective Strategies through Toll-Like Receptors

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Cerebral ischemic injury is one of the nation's leading causes of morbidity and mortality. Numerous patients at high risk of ischemic injury can be identified in advance and targeted for antecedent neuroprotective therapy—namely those undergoing planned cardiovascular interventions. A variety of preconditioning stimuli have been described that induce tolerance to ischemic brain injury in mouse stroke models, including Toll-like receptor (TLR) ligands. We have shown that systemic preconditioning with TLR9 ligand, CpG, induces significant neuroprotection against cerebral ischemia, through adaptive reprogramming of the response to ischemia. TLR9 is expressed on several cell populations systemically and in the brain. However, the site of receptor engagement and the molecular mechanisms required for protection are incompletely understood. Using a chimeric mouse model in which TLR9 is expressed selectively in specific compartments, we found that CpG-TLR9 engagement is required on BOTH circulating leukocytes AND parenchymal cells (e.g. endothelial, neurons, glial). The requirement for multiple cell types suggests a complex interplay between the circulation, where the drug is initially administered and the ultimate target, the brain. Using advanced imaging we found that CpG induces leukocyte-endothelial interactions that may contribute to protection. In addition, real-time imaging indicates that systemically administered CpG extravasates from the vessel into the brain parenchyma where it may target TLR9 expressing resident cells such as neurons, microglia and astrocytes, which could protect the brain directly. We have extended these studies to our novel non-human primate model of stroke where we have shown that preconditioning with CpG, substantially protected rhesus macaques against a subsequent cerebrovascular ischemic event. This is the first evidence of successful pharmacological preconditioning against cerebral ischemia in two species, mouse and non-human primate. The latter species offers significant promise for human applications. Thus our findings demonstrate the therapeutic potential of TLR ligands as powerful preconditioning agents,

capable of unleashing endogenous neuroprotective programs to protect against ischemic injury.

Dietary Preconditioning Limits Neurological Impairment in Stroke Models

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Intermittent fasting attenuates neurological damage in numerous disease conditions, including in cerebral ischemic stroke, where blood flow is transiently restricted to part of the brain, causing shortages of oxygen and glucose in the affected region. Although the mechanisms for dietary preconditioning are still under investigation, extended periods of fasting and re-feeding increase sensitivity to satiety signals, such as the adipocyte hormone leptin. Leptin-induced phosphorylation of signal transducer and activator of transcription 3 (STAT3) protects neurons in ischemia models, but the question of whether intermittent fasting (IF) recruits leptin-STAT3 signaling to minimize disruption of neuronal circuits after stroke has never been addressed. We maintained mice on IF or ad libitum feeding (AL) for three months to examine whether dietary neuroprotection is mediated by improved neuronal leptin sensitivity. These studies revealed that IF prevents reductions in circulating leptin after transient middle cerebral artery occlusion (tMCAO) and maintains synaptic integrity, assessed by quantifying dendritic spine density and synaptic protein expression. The protective effects of IF in vivo were recapitulated in vitro, where dietary preconditioning attenuated loss of dendritic spines following oxygen-glucose deprivation (OGD) in brain slices. Blockade of leptin-STAT3 signaling prevented neuroprotection in slices from IF mice, while leptin application protected against OGD-induced dendritic spine loss in slices from AL mice. Acute synaptic protection observed in IF mice after tMCAO was followed by attenuation of cell death and compensatory neurogenesis at later time points, suggesting that preventing circuit dysfunction immediately after an ischemic event limits subsequent neuronal loss. Taken together, these data indicate that IF maintains synaptic function by enhancing neuronal leptin sensitivity, with subsequent protection against cell death after an ischemic event. Stimulation of leptin-STAT3 signaling may therefore represent a potential strategy to limit circuit disruption and promote neuronal survival after stroke.

Microglia Regulate Blood Clearance in Subarachnoid Hemorrhage by Heme Oxygenase-1

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Subarachnoid hemorrhage (SAH) carries a 50% mortality rate. The erythrocytes that coat the brain contain heme that functions as a potent danger molecule resulting in sterile tissue injury and organ dysfunction. Free heme is metabolized via the inducible heme oxygenase-1 (*Hmox1*, HO-1) gene. Carbon monoxide (CO), a product of heme degradation by HO-1, is a bioactive gas with potent immunomodulatory properties. Here we demonstrate in a murine model of subarachnoid hemorrhage that HO-1 specifically expressed in microglia is necessary to attenuate neuronal cell death, vasospasm, impaired cognitive function, and controlled clearance of blood. Administration of CO initiated after SAH can rescue the absence of microglia HO-1 function to reduce injury by enhancing erythrocyte phagocytosis and does so through a reactive oxygen species and AMP kinase-dependent mechanism. In correlative human data, we find that patients with SAH have significantly higher HO-1 activity in cerebrospinal fluid (CSF) and peripheral blood compared to patients with unruptured cerebral aneurysms. Further, cisternal hematoma volume correlated with HO-1 activity in these patients measured by the presence of bilirubin. Collectively, we find that HO-1 and CO specifically in microglia are essential for blood clearance after SAH that otherwise results in neuronal injury and cognitive dysfunction.

CARDIOVASCULAR SESSION

Nitrite Mediates Delayed Protection through Modulation of Mitochondrial Function

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Cytoprotective Actions of Hydrogen Sulfide in Cardiovascular Disease

David J. Lefer, Louisiana State University, New Orleans, LA

Protecting the Heart with Exercise

John Calvert, Emory University, Atlanta, GA

Chemical and Pharmacological Preconditioning of Heart against Injury from Ischemia: Importance of the Dose-Response Relationship

John E. Baker, Medical College of Wisconsin, Milwaukee, WI

Cardioprotection with Ischemic Conditioning: The Comorbidity Conundrum

Karin Przyklenk, Wayne State University School of Medicine, Detroit MI
Peter Whittaker, Wayne State University School of Medicine, Detroit MI

Nitrite Mediates Delayed Protection through Modulation of Mitochondrial Function

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While nitrite was long thought to be physiologically inert, accumulating data demonstrates that the small anion is an endocrine store for nitric oxide and an intrinsic signaling molecule in its own right. Perhaps one of the most robust and reproducible effects of nitrite is its ability to mediate cardioprotection after ischemia/reperfusion (IR). Studies in various animal models have now shown that nitrite confers protection in a large temporal window that ranges from acute treatment during IR to one week prior to the ischemic episode. While this nitrite-mediated acute and delayed protection phenomenon has been observed by many labs, the subcellular mechanisms underlying nitrite's effect are still being elucidated. Mitochondrial dysfunction plays a central role in the pathogenesis of IR and modulation of mitochondrial bioenergetics is central to protection mediated by many preconditioning mimetics. Here we present data demonstrating that mitochondrial modulation is central to both the acute and delayed protection conferred by nitrite. Treatment with nitrite acutely, during IR, post-translationally modifies mitochondrial complex I to inhibit respiration, leading to an attenuation of mitochondrial reactive oxygen species and apoptotic signaling. However, administration of nitrite to normoxic cardiomyocytes induces mitochondrial fusion and augments mitochondrial oxidant production, leading to the activation of AMP Kinase, which mediates protection from a subsequent ischemic episode. These studies will be presented and the essential role of nitrite-mediated modulation of mitochondrial function will be discussed in the context of preconditioning.

Cytoprotective Actions of Hydrogen Sulfide in Cardiovascular Disease

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Although long considered a highly toxic gas known to be involved in industrial accidents, hydrogen sulfide (H_2S), has recently emerged as a critical physiological mediator of mammalian physiology. H_2S is produced endogenously at nanomolar to low micromolar concentrations in humans by the action of 3 enzymes including: cystathionine gamma lysase (CSE), cystathionine beta synthase (CBS), and 3-mercaptopyruvate sulfur transferase (3-MST). H_2S gas possesses a number of important physiological and biochemical properties that ultimately regulate cardiovascular homeostasis. H_2S is known to mediate vasodilation, inhibit inflammation, attenuate oxidative stress, modulate mitochondrial respiration, inhibit apoptosis, and regulate nitric oxide (NO) production. We have investigated the effects of both endogenous and exogenous H_2S on cardiovascular protection in the settings of acute myocardial infarction and heart failure in a number of preclinical model systems. We have previously reported that administration of H_2S donors and genetic overexpression of CSE protects the ischemic myocardium against infarction and preserves left ventricular function in the setting of heart failure in murine models. We have also demonstrated that genetic deficiency of CSE exacerbates hepatic and myocardial ischemia/reperfusion injury and promotes the severity of congestive heart failure. Recently, we have reported that H_2S activates endothelial nitric oxide synthase (eNOS) to increase nitric oxide (NO) bioavailability and thereby exert cytoprotective actions in disease states. Clinical studies are currently underway to evaluate the potential therapeutic benefit of H_2S releasing agents in the setting of heart failure. Taken, together our data strongly suggest that physiological or pharmacological levels of H_2S protect the heart and circulation against injury under basal conditions and during various disease states.

Protecting the Heart with Exercise

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Exercise training confers sustainable protection against ischemia-reperfusion injury in animal models and has been associated with improved survival following a heart attack in humans. It is still unclear how exercise protects the heart, but it is apparent that endothelial nitric oxide synthase (eNOS) and nitric oxide (NO) play a role. An additional question that remains to be fully answered relates to the molecular mechanisms that lead to the activation of eNOS during exercise. Recently, stimulation of the β_3-adrenergic receptor (β_3-AR) has been linked to eNOS activation and NO generation. However, the role that β_3-ARs play in mediating the cardioprotective effects of exercise is not fully understood. Here, we investigated the role of β_3-ARs, eNOS activation, and NO metabolites (nitrite and nitrosothiols) in the sustained cardioprotective effects of exercise. We found that voluntary exercise reduced myocardial injury in mice following a 4-week training period and that these protective effects could be sustained for at least 1 week following the cessation of the training. The sustained cardioprotective effects of exercise were mediated by alterations in the phosphorylation status of eNOS (increase in serine 1177 and decrease in threonine 495) leading to an increase in NO generation and storage of NO metabolites (nitrite and nitrosothiols) in the heart. Further evidence revealed that the alterations in eNOS phosphorylation status and NO generation were mediated by β_3-AR stimulation and that in response to exercise a deficiency of β_3-ARs led to an exacerbation of myocardial infarction following ischemia-reperfusion injury. Additional studies revealed that the increased level of myocardial infraction in these mice was likely due to eNOS uncoupling and increased NOS-dependent superoxide production. Our findings clearly demonstrate that the stimulation of β_3-ARs during exercise protects the heart against ischemia-reperfusion injury by increasing the cardiac storage of nitrite and nitrosothiols.

Chemical and Pharmacological Preconditioning of Heart against Injury from Ischemia: Importance of the Dose-Response Relationship

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Evaluation of a candidate agent for protection of the heart against injury from ischemia/reperfusion requires an experimental design that determines the dose-response relationship. Retrospective analysis of studies from the authors' laboratory revealed chemical pre-exposure conditioning with calcium, protons, gadolinium or nitrite, and pharmacological pre-exposure conditioning with protease activated receptor-1 and -4 antagonists, erythropoietin or thrombopoietin all exhibited dose- and concentration-dependent relationships for protection of the heart that are biphasic. There was no evidence of a linear dose response for cardio protection. A biphasic dose-response relationship was present for all *in vivo* and *in vitro* studies of cardio protection.

Thrombopoietin was initially identified as a cytokine and is currently used clinically to stimulate platelet production. When administered intravenously under novel pharmacokinetic parameters, thrombopoietin confers highly protective properties against cardiac injury arising from ischemia/reperfusion events without affecting platelet production at a dose that is around 20 times lower than currently used clinically. When administered at a higher dose that stimulates platelet production, thrombopoietin does not protect the heart against injury from ischemia/reperfusion. Thrombopoietin confers immediate concentration-dependent protection to human cardiac myocytes and rat hearts against injury from necrosis and apoptosis, and may offer long-lasting benefits. The underlying mechanism involves receptor-operated activation of cell survival pathways and activation of the ATP-dependent potassium channel. Inhibition of cell survival pathways and blockade of the ATP-dependent potassium channel abolishes pharmacological protection by thrombopoietin. The discovery that thrombopoietin confers dose- and concentration-dependent protection to organs in the context of acute ischemic events such as myocardial infarction indicates the potential for translation to human. The biphasic dose-response for thrombopoietin and other chemical- and pharmacologic-conditioning agents, characterized by low-dose protection and high-dose inhibition, indicates hormesis is present and is necessary for cardio protection to be manifest.

Cardioprotection with Ischemic Conditioning: The Comorbidity Conundrum

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The development of novel interventions capable of reducing damage to the heart by acute myocardial infarction (MI) or 'heart attack' remains a major, unmet clinical need. A wealth of preclinical evidence obtained in multiple models and species has established that ischemic 'conditioning' (encompassing the paradigms of preconditioning, postconditioning and remote conditioning) is profoundly cardioprotective, and has positioned the phenomenon as the most potent and promising candidate for clinical translation identified to date. However, the vast majority (>98%) of these experimental studies have been conducted using young, healthy adult cohorts: i.e., models that do not incorporate the risk factors and comorbidities associated with cardiovascular disease and acute MI in humans. Of potentially greater concern, emerging data suggests that comorbid conditions including diabetes and aging – and many of the drugs prescribed as standard of care in these patient populations – attenuate the expression and activity of key 'survival' kinases implicated to play a mechanistic role in conditioning-induced cardioprotection. A greater understanding of the consequences of clinically relevant comorbidities on the conditioned phenotype is essential for the future, successful exploitation of ischemic conditioning in MI patients.

BIOMEDICAL AND ENVIRONMENTAL SESSION

Cancer: A Metabolic Disease with Metabolic Solutions
Thomas Seyfried, Boston College, Boston, MA

Adaptive Homeostasis, Oxidative Stress, and Aging
Kelvin Davies, University of Southern California, Los Angeles, CA

Low-dose Radiation and Diabetes, and its Complications
Lu Cai, University of Louisville, Louisville, KY

Bystander Effects, Adaptive Responses and Hormesis: Being on the Right Part of the Stress Response Curve
Carmell Mothersill, McMaster University, Hamilton, ON, Canada
Colin Seymour, McMaster University, Hamilton, ON, Canada

Extracellular Oxidized DNA: A Novel Stressor with Hormetic Potential
Ancha Baranova, George Mason University, Fairfax, VA
Natalia Veyko, Research Centre for Medical Genetics, Moscow, Russia

Cancer: A Metabolic Disease with Metabolic Solutions

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Emerging evidence indicates that cancer is primarily a metabolic disease involving disturbances in energy production through respiration and fermentation. Numerous experiments in a broad range of organisms have shown that the hallmark feature of cancer, i.e., the unregulated growth of tumor cells, is suppressed following transfer of the tumor cell nucleus to cytoplasm of normal cells containing normal mitochondria. These findings indicate that nuclear genetic abnormalities alone cannot be responsible for cancer despite the commonly held beliefs of the field that cancer is a genetic disease. The genomic instability observed in tumor cells and all other recognized hallmarks of cancer are considered downstream epiphenomena of the initial disturbance of cellular energy metabolism. The disturbances in tumor cell energy metabolism can be linked to abnormalities in the structure and function of the mitochondria. Cancer growth and progression can be managed following a whole-body transition from fermentable metabolites, primarily glucose and glutamine, to respiratory metabolites, primarily ketone bodies. This transition will reduce tumor vascularity and inflammation while enhancing tumor cell death. A novel “press-pulse” therapeutic strategy is in development for the non-toxic metabolic management of cancer. Malignant brain cancer in preclinical models and humans will be used to illustrate general concepts. As each individual is a unique metabolic entity, personalization of metabolic therapy as a broad-based cancer treatment strategy will require fine-tuning to match the therapy to an individual’s unique physiology.

Adaptive Homeostasis, Oxidative Stress, and Aging

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Oxidative stress levels change continuously, depending on environment, metabolism, foods, drugs and medications, and radiation. Intracellular proteins are major targets of oxidative stress and cells must rapidly and selectively degrade mildly oxidized proteins, before they undergo more severe oxidation, aggregation, and cross-linking. Mammalian cells can readily adapt to mild (non-damaging) increases in oxidants signals, such as hydrogen peroxide, so that they become (temporarily) much more resistant to oxidative damage: 'Adaptive Homeostasis.' Such adaptive responses include the immediate disassembly of the 26S proteasome (catalyzed by HSP70 and Ecm-29) to form free 20S proteasome and 19S regulator complexes, at which point ATP/Ubiquitin-dependent proteolysis is temporarily lost. The additional free 20S Proteasomes (+/- 11S or PA28 regulators) are of immediate help in degrading oxidized proteins. The original 26S Proteasomes are re-assembled, and ATP/Ubiquitin-dependent proteolysis is restored over a three-hour period. During this three-hour period, and the subsequent 17 hours, new 20S Proteasomes, Immunoproteasomes, 11S (PA28) proteasome regulators, and mitochondrial Lon Proteases are synthesized, partially under the control of the Nrf2 signal transduction pathway. We have now obtained similar findings in *C. elegans* worms, and *D. melanogaster* fruit flies. Proteasome and Immunoproteasome subunit genes, the 11S (PA28) regulator, and Lon are true shock/stress genes that provide significant oxidative stress protection. In older cells, flies, and worms, Proteasome and Lon activities decline, and adaptational responses mediated by Nrf2 become ineffectual. The antagonistic effects of Bach1, c-Myc, and Nrf1 may confound adaptive homeostasis in older cells and organisms. Declining Proteasome and Lon activities and, perhaps, declining responsiveness to stress in general, may contribute to the ageing process, and to various age-associated diseases. Similar observations with other shock/stress genes/proteins have caused us to reconsider the Free Radical Theory of Aging, and we now propose that declining shock/stress gene inducibility may severely limit adaptive homeostasis in the elderly.

Low-Dose Radiation and Diabetes, and its Complications

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There remains lack of an efficient therapeutic, particular a non-invasive, approach to diabetic cardiovascular complications. Induction of hormesis and adaptive response by low-dose radiation (LDR) has been extensively reported. LDR-induced adaptive response makes the cells or tissue resistant to the damage caused by not only subsequently high-dose radiation, but also other non-radiation challenges such as diseases-related oxidative damage. Oxidative stress is a major cause for diabetic complications. Recently we have demonstrated the preventive or therapeutic effect of LDR on diabetic complications, including diabetic nephropathy, cardiomyopathy and wound healing impairment, in type 1 diabetic mice and rats that were induced with streptozotocin. However, these previous studies have mainly used 50 – 75 mGy whole-body X-irradiation daily for 8 – 16 weeks, whether such exposure conditions are the optimal conditions to protect the kidney without significant toxic effect on normal tissues or animals have not been addressed yet. In our recent studies, we have examined the preventive effects of different exposure conditions, including different dose levels (12.5, 25 and 50 mGy), exposure frequencies (daily, every other days, and every week), and exposure modes (in whole-body or renal region only) on the renal functional and pathogenic changes of diabetic mice. Diabetic mice were induced with multiple low-dose streptozotocin protocol. These studies demonstrate that LDR as a non-invasive approach has a great potential to be considered a new alternative therapy for the senior diabetic patients with significant renal dysfunction for whom the routine medication can not be given due to renal failure to eliminate their metabolisms.

Bystander Effects, Adaptive responses and Hormesis: Being on the Right Part of the Stress Response Curve

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The dose response relationship for ionizing radiation is very controversial. There is assumption in radiation protection of a linear dose response and a single dose dependent DNA strand break mechanism. Despite many studies showing adaptive, hormetic and various inducible responses, the DNA centric paradigm dominates. The latest data from our laboratory strongly suggest that the low dose mechanisms are completely independent of mechanisms operating at higher doses and may not even be temporally related. We have found that a low dose delivered after a high dose can produce the same type of adaptive response as is seen when the low or conditioning dose is given first. Important endpoints at low doses are apoptosis and changes in gene or protein expression patterns, which often play out as hormetic, protective and adaptive effects rather than harmful or potentially carcinogenic effects. Genotype is important in determining outcome as are system level micro and macro environmental effects. While targeted doses are kept within accepted safety limits, little is known about the non-targeted effects (NTE) of exposure such as the role of bystander effects in mediating response to stressors. In particular it is unclear whether the radiation weighting factors and dose rate effectiveness factors used in radiation protection apply following non-targeted or low dose exposures. The literature is confusing and controversial. Low energy protons and neutrons do not appear to produce NTE, although high energy protons, alpha, beta and gamma radiations do and NTE have also been reported following UVA/B exposures. The dose responses for NTE typically saturate around 0.5Gy acute dose and have a low dose threshold for induction of only a few mGy. They should therefore be expressed at doses typically used in nuclear imaging and in targeted therapy. We suggest that understanding the factors controlling low dose effects and in particular the post-conditioning effect could be critical to resolving the debate about the benefits or dangers of radiation exposure.

Extracellular Oxidized DNA: A Novel Stressor with Hormetic Potential

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Chronic stress appears to accelerate biological aging, and oxidative damage is an important potential mediator of this process. Many chronic diseases are accompanied by an increase in overall oxidation of genomic DNA, with the most common products being the thymidine glycol and 8-hydroxy-2'-deoxyguanosine (8-oxodG). In fact, the 8-oxodG is the most widely used "marker" for oxidative DNA damage. The cells with the most damaged DNA die either by necrosis or by apoptosis. The oxidized DNA released from the dying cells is likely the most prominent contributor to the pool of cell-free/ extracellular DNA. The highly oxidized cfDNA that circulates in patients with high oxidative stress levels may substantially add up to endogenous stress levels by direct and significant modulation of a varity of the physiological activities. It is however remarkable that the treatment of human cells with relatively small amounts of oxidized DNA evokes an adaptive response. For example, an increase in the rates of survival after the treatment with low amounts of oxidized DNA was shown both in serum starving cell populations and in the cell irradiated at dose of 1.2 Gy. Similar effects were observed in mesenchymal stem cells (MSCs). The latter observation has direct therapeutic relevance as the preconditioning of MSCs by exposure to low concentrations of oxidized DNA fragments would probably augment their survival rate in pathological microenvironment of diseases tissues. It should be also noted that the presence of the high amounts of endogenous oxidized DNA in bodily fluids of patients with chronic diseases may interfere with therapeutic potential of introduced MSCs. Effects of exposure to oxidized cfDNA should be taken into account when treating tumors with various ROS-producing agents and irradiation. The damaged DNA released from irradiated cells may be responsible for previously unexplained abscopal effects of local irradiation.

APPLICATIONS AND PERSPECTIVES SESSION

Clinical Applications of Pre-, Post- and Remote Ischemic and Pharmacologic Conditioning in Ischemic Disease: Promise and Limitations

Ronald J. Korthuis, University of Missouri, Columbia, MO

Exercise-Induced Preconditioning in Cardiac and Skeletal Muscles

Scott K. Powers, University of Florida, Gainesville, FL

Preconditioning Can Modulate Therapeutic Index of Photobiomodulation

Praveen Arany, National Institutes of Health/NIDCR, Bethesda, MD

The Case for Hormetic Dose-Response and against the LNT

Richard A. Williams, Mercatus Center at George Mason University, Arlington, VA
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Clinical Applications of Pre-, Post- and Remote Ischemic and Pharmacologic Conditioning in Ischemic Disease: Promise and Limitations

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The aim of this presentation is to summarize perspectives on the potential use of conditioning strategies to reduce the extent of tissue injury induced by ischemia/reperfusion (I/R). Both acute and delayed phase ischemic preconditioning (IPC) may be used in cases where the onset of ischemia can be anticipated, such as prior to cardiopulmonary bypass or organ transplantation. While induction of IPC in internal organs in other ischemic conditions is technically challenging in patients, the finding that subjecting limbs to IPC provokes a protected phenotype in distant organs provides a clinically suitable approach. The discovery of ischemic postconditioning, or staccato reperfusion, was a landmark finding not only because it established reperfusion as an iatrogenic cause of injury, it also opened up the realm of pharmacologic postconditioning to limit tissue injury a period of unforeseen ischemia. Indeed, mechanistic identification of the underpinning signaling steps mediating ischemic conditioning has been aggressively pursued to identify pharmacologic strategies for clinical use, raising the hope that tissues could be chronically preconditioned as a therapeutic strategy to decrease the extent of injury that would otherwise occur in response to an unanticipated adverse cardiovascular event. Moreover, pharmacologic preconditioning of stem cells has been proposed as a means to maintain their viability in harsh postischemic tissue environments. Gene therapy approaches are also being investigated as a means to permanently upregulate the expression of proteins identified as protective in conditioning studies, thereby invoking the appearance of a sustained defensive phenotype. Disappointingly, the clinical utility of conditioning strategies is limited by the development of tachyphylaxis and because these approaches are ineffective in the presence of co-existing risk factors. However, antecedent ingestion of alcoholic beverages induces the appearance of a protected phenotype that does not show densitization over time and remains effective in presence of co-morbidities, pointing the way for the development of new treatment modalties. Supported by NIH grant AA014945.

Exercise-Induced Preconditioning in Cardiac and Skeletal Muscles

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Regular sessions of endurance exercise training have been shown to modify protein expression in both cardiac and skeletal muscle fibers. This exercise-induced preconditioning in cardiac and skeletal muscle fibers results in an altered fiber phenotype that resists cellular damage in response to a variety of stresses including ischemia-reperfusion injury, chemotherapeutic drugs (e.g., doxorubicin), inactivity-induced skeletal muscle atrophy, and heat stress. The cellular mechanisms responsible for exercise-induced preconditioning have been the topic of investigations for over a decade. The goal of this presentation is twofold: 1) provide a state-of-the-art overview of the mechanisms responsible for exercise-induced cardioprotection against ischemia-reperfusion injury; and 2) to discuss the mechanism(s) accountable for exercise-induced skeletal muscle protection against doxorubicin-induced myopathy and inactivity-induced muscle atrophy. The conversation will conclude with a discussion of how studies into the mechanism(s) responsible for exercise preconditioning in myocytes can provide the foundation for new therapeutic strategies in the prevention of stress-induced myocyte damage.

Preconditioning Can Modulate Therapeutic Index of Photobiomodulation

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Photobiomodulation (PBM, also known as Low level light therapy-LLLT) has been around for many decades and the invention of the laser prompted more intense investigation into its therapeutic benefits. The lack of biological mechanisms and complexity of technology has prevented the field from more mainstream acceptance. Significant advances in optics and photonic technologies along with recent insights into molecular mechanisms have provided promising tangible progress towards mainstream acceptance of this therapy. A major limitation currently is the use of 'low' doses that are either inefficacious (no response) or provide very subtle biological responses. On the other hand, higher power (dose) lasers are exquisite photodynamic therapy and surgical tools and can cause severe tissue damage. Hence, we have been attempting to define the therapeutic threshold where the low, therapeutic laser PBM dose can be differentiated from the high, detrimental PDT laser doses for their safe and effective clinical use. Interestingly, we have observed that preconditioning with low, but not high, amounts of a stressor can potently alter the damage threshold. We have characterized the cell stress response pathway involving endoplasmic stress that is primed by the precondition pre-stress. The ER stress pathway orchestrates the cellular decision to repair the sub-lethal damage or commits the cell to undergo cell death via apoptosis. This presentation will outline the molecular pathways involved in laser phototoxicity and the clinical implications of preconditioning that could alter therapeutic index for PBM therapy.

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The Case for Hormetic Dose-Response and Against the LNT

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For years, the linear no threshold (LNT) model has been the favored dose-response model for chemicals. This paper will address three reasons why the LNT model should be abandoned as the default model in risk assessment. First, it is not possible to validate LNT predictions via toxicological and epidemiological methods at exposures relevant to the general public. The LNT model ignores the effects of the range of adaptive processes such as DNA repair, apoptosis, preconditioning/adaptive responses, and hormesis. Large numbers of experimental studies contradict predictions in the chemical toxicology and ionizing radiation areas. Second, risk is ubiquitous and even risk reducing actions can have a risk increasing reaction. Moving to lower and lower doses of a targeted compound can increase risk by additional exposure to substitute compounds in a manner that can exceed any risk reduction from reducing exposure to the targeted compound. Finally, due to diminishing marginal returns, the lower the mandated exposure, the higher the cost. As regulatory costs are paid by taking resources out of consumers' hands, some portion of those costs would have been used to reduce private risks. This effect can also drive up risk on balance. Together, these effects suggest there should be no default model in risk assessment, and that using the LNT model as a default option is in no way conservative." Instead, risk assessors should use whatever model is most supported by the evidence.

POSTER SESSION

Post-Conditioning Stress (PCS) Responses in HaCaT Cell Line and a Comparison between PCS with the Adaptive Stress Response

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Opposite Bystander Effect Induced by the Low-Dose Hyper-Radiosensitive Region in C6 and F98 Rat Glioma Cell Lines

Cristian Fernandez-Palomo, McMaster University, Hamilton, ON, Canada
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Association between Air Temperature and Circulatory System Disease Mortality in New England Counties

John Hart, Sherman College of Chiropractic, Spartanburg, SC

Measures for Monitoring Adaptability of the Nervous System

John Hart, Sherman College of Chiropractic, Spartanburg, SC

Low-Level Laser Therapy for Neuromusculoskeletal Conditions: A Mini-Review

Lucian Henry, Prime Care, Greenville, SC

The Use of X-rays in the Treatment of Bronchial Asthma: An Historical Assessment

Edward J. Calabrese, University of Massachusetts Amherst, Amherst, MA
Gaurav Dhawan University of Massachusetts Amherst, Amherst, MA
Rachna Kapoor, University of Massachusetts Amherst, Amherst, MA

One Dose...Many Consequences

David Kirkland, Ottersgill, Stromness, Orkney, UK

Chernobyl-Related Cancer: On the Role of Late Diagnostics in the Incidence Increase

Sergei V. Jargin, Peoples' Friendship University of Russia, Moscow, Federation of Russia

Hormesis: General Principle only for the Environmental Agents

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Radiation-Stimulated Ultraviolet Signal Generation and Response by Various Cell Lines

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Fiona McNeill, McMaster University, Hamilton, ON, Canada
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Andrew J. Rainbow, McMaster University, Hamilton, ON, Canada
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Astrocytes Remain Neuroprotective even after Severe Stress and Loss of Glutathione Defenses

Rehana K. Leak, Duquesne University, Pittsburgh, PA
Amanda M. Gleixner, Deepti B. Pant, Jessica M. Posimo, Duquesne University, Pittsburgh, PA

Beneficial and Neutral Effects of Radiation: Data Gaps in Radiobiological Literature

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Colin Seymour, McMaster University, Hamilton, ON, Canada
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Estimation of Lifetime Risk of Cancer with Long-term Survival Rates from Radiation Exposure

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Eun-Kyeong Moon, Korea University, Seoul, South Korea
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Microarray Analysis for Breast Cancer Cells with the Decreased Malignant Properties by Low-Dose Radiation

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Deposition of ^{226}Ra in Fish Fed with Environmental Relevant Activities of ^{226}Ra

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Post-Conditioning Stress (PCS) Responses in HaCaT Cell Line and a Comparison between PCS with the Adaptive Stress Response

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Postconditioning Stress (PCS) in physiology refers to a small exposure given after an adverse outcome due to the same stressor , leading to improvements in outcome. The mechanisms are not clear but the phenomenon is clearly of interest because it departs from the linear dose response thinking which dominates biology. In radiobiology, PCS refers to subjecting cells to a large radiation dose, followed by exposure to a subsequent smaller dose - i.e. a reverse of the usual adaptive response. Previous data from our group using high doses suggested that both pre and post conditioning stress could modify the dose response but the experiments were not followed up. In the current experiments, an immortalised line of human keratinocyte cells, HaCaT cells, was seeded at clonogenic cell densities (about 400 cells), and these were irradiated using the McMaster Taylor Source. This source consists of a 1 kCi Cs-137 *β^- emitter, from which a 661.675 keV* γ (gamma) ray is also emitted 85.1% of the time. Cells were exposed to an initial dose of 4Gy. After 3hrs a second dose of 100mGy was delivered. Controls were unirradiated cells, cells receiving 4.1Gy total dose in one go and cells receiving the lose dose before the high dose. The experiment was repeated with a first dose of 0.5Gy and a post conditioning dose of 5mGy using appropriated controls as these are important mechanistic dose points in radiobiology. The results suggest non-linear pre and post conditioning adaptive responses. The data have implications for our understanding of low dose radiobiological mechanisms.

Opposite Bystander Effect Induced by the Low-Dose Hyper-Radiosensitive Region in C6 and F98 Rat Glioma Cell Lines

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Previous studies involving animals harbouring C6 and F98 tumours have shown contradictory results. While tissue explants from rats harbouring C6 glioma induced stronger bystander effects than tumour-free animals, explants from rats harbouring the F98 tumour produced weaker bystander effects than their tumour-free irradiated controls. These results made us wonder what is the nature of the different effects and to which extent the two tumour cell lines cause these. The aim of this study was to investigate the inter-relationship between the response of C6 and F98 rat glioma to low doses and their ability to induce bystander effects.

Each C6 and F98 cell line was arranged in three different groups. The directly irradiated (group 1) and the donor (group 2) received 12 different gamma-ray doses between 0-3 Gy with a focus on the low-dose hyper-radiosensitive region (<1Gy). Group 1 were used for study of the direct effect of radiation using a clonogenic assay. Group 2 served as donors of bystander signals, which were secreted into culture media. This was harvested, filtered and transferred onto reporter cells (group 3) for the study of radiation-induced bystander effects using a clonogenic assay.

Our results show that both cells lines are hyper-radiosensitive at low doses and they show increased radioresistance as the dose is increased. However while F98 bystander signals induced a decrease in survival in their reporters, C6 bystander signals induced an increase in survival in theirs. We speculate that the differing p53 status of the cell lines may underlie this difference.

We conclude that the C6 and F98 rat gliomas can effectively produce bystander effects at low doses, but while the C6 stimulated survival, F98 induced cell death. These results suggest two different mechanisms for the low-dose effects in these rat glioma cell lines, which is in accordance with our previous findings in animal models.

Low-Dose Serotonin and Histamine Therapy Increases Tetanic Forces of Human BioArtificial Muscles, Reduces Muscle Injury, and Improves Grip Strength Performance of mdx Mouse

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Duchenne muscular dystrophy (DMD) is a progressive muscle degenerative disorder caused by a mutation in dystrophin gene. Even though several therapeutic approaches have been studied, none has led to substantial long-term effects in patients. The aim of this study is to test low-dose serotonin and histamine therapy (S&H) on human skeletal myoblasts and mdx animal model for its effects on muscle strength and injury. Normal human BioArtificial muscles (BAMs) were treated, muscle strength and injury tests were conducted using MyoForce Analysis System (MAFS^{TM}). Murine model of DMD, Dmd^{mdx} mice, was treated twice daily for six weeks and muscle performance tests were conducted once weekly. Low-dose serotonin and histamine treatment showed significant increase in muscle tetanic forces at all time-points and concentrations compared to saline treated control. Dose response of the BAMs to the treatment demonstrated a significant increase in force generation on day 3 and 4. While low-doses of S&H increased muscle strength, highest two doses had a significant effect on reducing injury to the mBAMs as measured by a reduction in release of adenylate kinase. S&H combination and histamine alone therapy improved grip strength of Dmd^{mdx} mice while serotonin alone treatment resulted in no significant improvement in muscle strength. The results of this study demonstrated that low-dose serotonin and histamine therapy might play a key role in potential treatment for muscular dystrophy and its clinical potential should be further evaluated.

Key words: duchenne muscular dystrophy, histamine, serotonin, muscle strength, bioartifical muscles, mdx mouse.

Association between Air Temperature and Circulatory System Disease Mortality in New England Counties

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There is concern in recent years about "global warming," characterized by the EPA as an increase in global temperature of about 1 degree F. However, little actual data is available to justify the concern. The present ecological study tested global warming theory on a local level, in the six states that comprise New England: ME, NH, VT, MA, RI, and CT. Air temperatures by county were compared to circulatory system disease mortality (CSDM) by county. Since low level radiation has been linked to heart disease mortality, a proxy variable for cosmic radiation - land elevation - was also included in the analysis. Another reason for including land elevation is because it tends to be (inversely) related to air temperature. For the 67 New England counties, average daily maximum air temperature (for 2011 – the most recent year available at time of the study) and CSDM (also for 2011) were obtained from interactive CDC databases. Land elevation data for the geographic center of each county were obtained from an interactive U.S. Geological Survey database. To control for the factor of race, a single race was selected, one where most counties reported data (whites). Statistically significant correlations (two-tailed $p \leq 0.05$) or those nearly-so, were included in multiple linear regression to determine their relative strength of association with the response variable (of CSDM). Weak correlations were observed between CSDM and: a) temperature ($r = -0.217$, $p = 0.08$); and b) land elevation ($r = 0.265$, $p = 0.03$). In linear multiple regression, these predictors (temperature and land elevation) cancelled each other out and were rendered statistically insignificant. Thus, in this study, no adverse association was observed between warmer air temperatures and circulatory system disease mortality. Further study is indicated for other outcomes and geographical areas in an effort to study the effects (or lack thereof) from "global warming."

Keywords: Global warming, mortality, New England

Measures for Monitoring Adaptability of the Nervous System

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Adaptability is one of the key themes in the International Dose Response Society's conference on Preconditioning in Biology and Medicine. In many clinical investigations on adaptive responses in humans, long periods of time are typically required to determine what types of adaptation, if any have occurred. For example, a comparison of morbidity rates between a group that received an exposure of a stressor (e.g., low level radiation) or intervention (e.g., chiropractic adjustment or a pre-conditioning procedure) to a group that was unexposed / did not receive the intervention may require months or years of follow-up. Research clinicians however, may be interested in obtaining evidence of adaptive responses (or lack thereof) sooner than this, perhaps even minutes following the exposure or intervention. Since the autonomic nervous system plays a key role in adaptive processes, an autonomic biomarker would have an appropriate application in this regard. One such test would be heart rate variability, where greater variability represents greater adaptability and better clinical outcomes, compared to lower heart rate variability. One limitation to heart rate variability is that it may not be available or feasible in some settings. Alternatively, the more user-friendly method of resting pulse rate has been shown to have a good correlation with heart rate variability. A lower resting pulse rate tends to be associated with higher heart rate variability and better clinical outcomes. Thus, resting pulse rate can be viewed as a proxy assessment of autonomic adaptability. Consequently, heart rate variability, and the more user-friendly method of resting pulse rate, may be helpful options for assessing autonomic adaptability immediately following exposure or intervention.

Keywords: Biological adaptation, heart rate control, pulse rate

Low-Level Laser Therapy for Neuromusculoskeletal Conditions: A Mini-Review

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Low-level laser therapy has been shown to have a biphasic dose response. Laser photobiostimulation accelerates tissue healing, reduces inflammation and provides analgesia. Proposed mechanisms include increased nociceptive threshold, blocking nerves through axonal flow or enzyme inhibition, increased endorphin production, COX-2 inhibiition and attenuation of prostaglandin-2, and vasodilation. Evidence of efficacy exists for temporomandibular joint disorder, knee osteoarthritis, diabetic neuropathy, myofascial pain, tendinopathy, chronic epicondylitis, low back pain and radiculopathy, carpal tunnel syndrome, lumbar disc herniation, frozen shoulder, tension headache, Raynaud's phenomenon, fibromyalgia, injury resulting from sports or motor vehicle trauma. Studies on the treatment of specific neuromusculoskeletal conditions have had mixed results, with some indicating benefit and others showing it to be no more effective than placebo. Conflicting results in the existing literature can be attributed to suboptimal wavelength, amplitude, and dose and the skill and experience of the operator. Low-level laser therapy is a safe and effective modality for many neuromusculoskeletal conditions and should be considered by the clinician for inclusion in a multimodal treatment plan. Further research is encouraged to determine the underlying physiological mechanisms and optimum treatment protocols.

Keywords: Low-level laser therapy, pain, musculoskeletal

The Use of X-rays in the Treatment of Bronchial Asthma: An Historical Assessment

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This historical assessment provides a review of the role of X-ray therapy in the treatment of bronchial asthma, which spanned the first six decades of the 20th century, involving nearly 6,000 patients in published clinical case studies. Patients selected typically had at least moderate to severe asthma and were refractory to other commonly employed treatments. The results of more than 60 studies indicated that about 70% of patients had rapid and marked reductions in clinical symptoms with about half of these patients showing complete symptom relief. The duration of the beneficial responses was variable but was approximately 1-6 months for about 50% of the benefited patients, and between 1 – 4 years for the upper 25% of patients. The use of X-rays in the treatment of such patients fell into disfavor during the 1950s due to mounting concerns with possible enhanced risks of cancer which coincided in time with the discoveries and use of anti-histamine medications, antibiotics and the methyl xanthine bronchodilators aminophylline and theophylline.

Key Words: asthma, X-rays, radiotherapy, radon, inflammation, anti-inflammatory phenotype, history of medicine

One Dose...Many Consequences

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The Linear No Threshold Hypothesis, cornerstone of radiation protection, yet supported by 'a falsified research record'[1], apparently endures because of its simplicity and the seemingly conservative nature of its risk prediction. But is it really conservative? What happens if we apply the hypothesis to those substances responsible for most of our dose, the internal alpha emitters, both natural (radon and polonium) and anthropogenic (plutonium)?

Continuous exposure to radon at 20Bq/m3 (UK average[2]) results in about ten alpha decays per second in our lungs, one in every 100 lung cells experiencing one alpha traversal a *year* and 1mSv/year effective dose equivalent (He^{3}). The anthropogenic dose, from alpha emitting particulates (ca. 0.001mSv annually from $Pu^{4\&5}$), would probably expose a few groups of lung cells to multiple alpha traversals every *day* causing clusters of dead and injured cells. Can a single quantity (He =1.001mSv) reliably represent the effects of such vastly different activity distributions? Doesn't homogeneity of alpha dose influence effects? The pharmaceutical industry has homogeneity standards; why doesn't the nuclear industry?

It has been argued, on the grounds of cell killing and number of cells at risk that, for particulate alpha sources, carcinogenic risk per unit dose should increase with source *fragmentation.* Experimental evidence using different plutonium isotopes and particle sizes supports this argument, indicating that greater dose homogeneity (greater fragmentation) does indeed increase carcinogenic risk. By extrapolation, it can be argued that homogeneity standards aren't needed because domestic radon and polonium, being monatomic, provide the maximum possible homogeneity and hence maximum risk per unit dose. With no detectable health issues from their 1mSv, aren't concerns about 0.001mSv from anthropogenic alpha emitters therefore unjustified? Scrutiny of early experimental results suggests not. The conclusion is 0.001mSv could be more carcinogenic than 1mSv! Extrapolation has misled us: the homogeneity/risk relationship is non- linear.

Referenc/es

1 E.J.Calabrese, Cancer risk assessment foundation unraveling: New historical evidence reveals that the US National Academy of Sciences (US NAS), Biological Effects of Atomic Radiation (BEAR) Committee Genetics Panel *falsified the research record* to promote acceptance of the LNT http://www.ncbi.nlm.nih.gov/pubmed/25600588 (January 2015, available by e-publication only)
2 Building Research Establishment UK: http://www.bre.co.uk/page.jsp?id=3133
3 British Geological Survey (NERC) UK Geohazard Note, Naturally occurring radon (May 2012) http://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=8&ved=0CFIQFjAH&url=http%3A%2F%2Fwww.bgs.ac.uk%2Fdownloads%2Fstart.cfm%3Fid%3D2498&ei=ImTLVO64Lcm7UZP3gbAB&usg=AFQjCNFbmx2JUd80e7Xu_pZ2jO-eGi5Ubw&bvm=bv.84607526,d.d24
4 P.J. Mango et al, Plutonium in environmental and biological media, Health Physics (1967)
5 Calculations based on above refs assuming lung burden of ca 0.3pCi (0.01Bq)

Chernobyl-Related Cancer: On the Role of Late Diagnostics in the Incidence Increase

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The reported incidence of thyroid cancer in children and adolescents in Soviet Union before the Chernobyl accident was lower than that in other developed countries. This is not clearly perceptible from the literature because comparisons of the high incidence figures 4 years after the accident and later have been made with those from the first years after the accident, when the registered incidence had already started to grow. Considering the low pre-accident registered incidence, there was an accumulated pool of undiagnosed tumors before the accident. The percentage of more advanced cancers, larger in size and less differentiated, was higher at an earlier time after the accident, when the pool of neglected cancers was diagnosed due to the screening and improved diagnostics. Some of these advanced tumors found by screening were interpreted as aggressive radiogenic cancers. The same tendency is apparently true also for other cancers, e.g. renal carcinoma. Furthermore, the screening-effect, false-positivity and registration of non-exposed patients as Chernobyl victims has obviously contributed to the registered incidence increase of malignancy after the accident (Dose Response 2014;12:404-14). Implications for the assessment of the dose-response pattern for low dose/dose rate ionizing radiation are discussed in the presentation.

Hormesis: General Principle only for the Environmental Agents

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Hormesis is a concept of biphasic dose-response to different pharmacological and toxicological agents. According to this concept, a small dose of a noxious agent can be beneficial. Certain publications generalizing hormesis (e.g. Biogerontology 2012;13:215) can be cited in support of homeopathy. Claims that homeopathy is based on hormesis create an illusion that it employs a scientific method. Low-dose impacts may be associated with an enhanced risk under conditions of organ subcompensation. Some noxious stimuli can act synergistically with other agents, for example, on the cells with a limited or no capacity for cellular regeneration such as cardiomyocytes or neurons in conditions of pre-damage e.g. by ischemia. In such conditions, which are not uncommon especially in elderly patients, the concept of hormesis can be dangerous if used in the clinical thinking and decision-making. For example, it would hardly be indicated to apply mild asphyxia in angina pectoris or small doses of ethanol in end-stage liver disease with a hope for a hormetic effect as a general biological dose-response pattern. Recommendations for practice should be based neither on hormesis as a default approach nor on the postulates of homeopathy. It cannot however be excluded that some empirical knowledge is used in homeopathy independently of her theoretic principles. Supposedly useful empirical knowledge should be discussed in the professional literature and tested by methods of evidence-based medicine. A hypothesis is discussed in this presentation that hormesis as a general principle can be assumed only for the factors that are present in the natural environment thus having induced adaptation of living organisms. Accordingly, existence of its optimal level can be assumed, which would correspond to the current environmental level or, considering that the natural selection is a slow process, to some average of historic levels. A priori generalizations of hormesis, encompassing substances and other factors that are not present in the environment, are unfounded. All clinically relevant effects, hormetic or not, should be tested by the methods of evidence-based medicine.

Radiation-Stimulated Ultraviolet Signal Generation and Response by Various Cell Lines

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We have recently shown that photon emission in the ultraviolet (UV) range occurs in cells exposed to tritium. We have linked this UV emission to the bystander effect in unirradiated cells receiving only photon emissions from irradiated cells. To investigate the potential influence of p53 upon UV signal generation and upon the response of bystander cells to the generated signals, 5 cell lines of various p53 statuses (HaCaT (partly mut), SW48 (wt), HT29 (mut), HCT116 p53+/+, HCT116 p53 -/-) were irradiated with beta-emitter, tritium,. Photon emission (340 nm±5nm) from irradiated cells was quantified using a photomultiplier tube and signal response (clonogenic survival) was tested by placing reporter cell flasks directly superior to irradiated signal-emitting cells. All cell lines were found to emit signals upon exposure to tritium. The magnitudes of HaCaT and HT29 photon emission at 340 nm were similar to each other while they were significantly different from the stronger signals observed from SW48, HCT116+/+ and HCT116-/- cells. Upon observing responses, HaCaT, HCT116+/+ and SW48 cells demonstrated changes in survival as a result of exposure to emission signals. HCT116-/- and HT29 cells did not exhibit any changes in survival and thus were considered unable to respond. The survival response was found not to correspond with the observed signal strength for all experimental permutations; this result may be attributed to varying emission spectra from cell line to cell line or differences in response sensitivity. Overall, these results indicate to us that a bystander response may be influenced by the p53 status of the cell line. Each of the cell lines exhibiting wild type p53 (HCT116+/+ and SW48) or at least some wild type activity (HaCaT has 3 wild-type codons in the binding site) demonstrated the ability to respond to the UV signal. However, the cells possessing null or mutated p53 did not respond to the signals from any of the cells tested. This leads to the suggestion that response to the ultraviolet signal that is emitted from tritium-irradiated cells is related to the cell line's p53 status.

Astrocytes Remain Neuroprotective even after Severe Stress and Loss of Glutathione Defenses

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Preconditioning is traditionally recognized as a protective response to subtoxic stress. However, we have shown that primary cortical astrocytes that manage to survive toxic challenges are also preconditioned against subsequent stressors, such as proteasome inhibitors (MG132) and oxidative toxins (paraquat). This suggests that the phenomenon of preconditioning can be extended from subtoxic to toxic stress. We have also found that inhibition of glutathione synthesis with buthionine sulfoximine abolishes astrocytic resistance to a second MG132 hit, suggesting that severe stress-induced preconditioning of astrocytes is glutathione-dependent. Here we tested the hypothesis that astrocytes preconditioned by severe stress could still protect neighboring neurons and that this effect was also dependent upon glutathione. In order to deliver severe stress, astrocytes were subjected to MG132 treatment at concentrations that killed ~50% of the population. The MG132 was then removed and primary cortical neurons were plated on top of the stressed astrocytes. The astrocyte/neuron cocultures were then exposed to another MG132 hit. Neurons that were plated on top of previously stressed astrocytes were better protected against MG132 than neurons plated by themselves. These results suggest that stressed astrocytes are still well prepared to protect nearby neurons from cell death. Surprisingly, inhibition of glutathione synthesis did not abolish the protective capacities of astrocytes, suggesting that the effect remains robust even under conditions of oxidative challenge. Taken together, these results support the view that severe stress can also be a preconditioning stimulus and that it can prime astrocytes to protect neighboring neurons under conditions of proteotoxic and oxidative injury.

Beneficial and Neutral Effects of Radiation: Data Gaps in Radiobiological Literature

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Datasets reporting the effects of ionizing radiation upon biota have largely ignored beneficial and neutral effect responses. This is a major concern as focusing on the negative effects of radiation may have an impact on the validity of guidelines and policies used for the radiological protection of biota. To fill this data gap, a literature review is being undertaken to identify studies reporting beneficial or neutral effects of radiation exposure. Given that biological responses of organisms to radiation are complex and the response of a single cell, or group of cells, cannot necessarily be extrapolated to explain the response of the organism as a whole, the review is focusing on studies at the organismal level. Preliminary results indicate that studies describing beneficial or neutral responses at this biological level are largely absent from the literature. Barriers preventing research at the organismal level and a bias towards the investigation of negative effects of radiation, compounded by outdated perceptions and the incapacity of biological models to handle adaptive effects, may be contributing to this data gap.

Estimation of Lifetime Risk of Cancer with Long-term Survival Rates from Radiation Exposure

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Assessment models to estimate lifetime cancer risk from radiation exposure are generally defined as functions of exposure dose, age at exposure, attained age, gender and study population-specific factors. Since the models were developed from dose-response data primarily using the Life Span Study cohort of Japanese atomic bomb survivors, it is necessary to transport risk estimates from the Japanese to target populations with baseline risks and life tables of their own countries. Life tables employed in most assessment models are based on all-cause mortality rates. Thus, this would be inadequate for estimating lifetime risk of cancer incidence if the cancer is not life threatening with a long-term survival rate. Thyroid cancer in South Korea, especially for women, has the highest incidence rate and the lowest mortality rate. The incidence rates per 100,000 are 28 for men and 120 for women in 2012, and the 10-year survival rates for both genders are over 95 percent. Here we present the lifetime attributable risk (LAR) estimates of thyroid cancer and all-solid cancer in South Korea for lifetime exposure to 1 mSv per year using the life tables based on cancer-free survival rates suggested in the recent Fukushima health risk assessment by the World Health Organization. Compared with the estimates of LAR based on all-cause mortality rates, LAR of thyroid cancer and all-solid cancer using cancer-free survival rates decreased by about 1% and 12 % for men and about 5% and 7% for women. However, the change of lifetime fraction risk (LFR) was less than 1%. Given the trend to increase of cancer incidence along with long-term survival rates, life tables solely based on all-cause mortality data may lead to overestimates of the lifetime risk of cancer incidence.

Microarray Analysis for Breast Cancer Cells with the Decreased Malignant Properties by Low-Dose Radiation

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Although there is an accumulating body of experimental data about ionizing radiation at less than 100 mSv, the physiological effects of low dose radiation (LDR) cannot be concluded as beneficial or detrimental to human. Compared with non-irradiated control, LDR irradiated condition shows a small difference with much variation in each experimental sample, except for some specific cases. Insufficient understanding of the molecular effects of low levels of radiation exposure has leaded to a great uncertainty regarding its health effects. In order to increase the consistency and coherence in LDR experimental data, lots of cellular and biochemical approaches have been attempted. In this study, we evaluated the fractionated and single irradiated LDR effects on the malignant properties of breast cancer cells. The conventional Boyden chamber assay was performed for two dimensional (2D) cultured cells to evaluate the migration and invasion ability of LDR irradiated breast cancer cells. We also examined the malignancy of LDR irradiated cells with the three dimensional (3D) culture systems, which is known to possess many features mimicking the *in vivo* biological entities. Moreover, LDR irradiated cells were investigated the sensitivity to several anti-cancer drugs. As a result, we found that both fractionated and single LDR irradiation decreased malignant properties of breast cancer cells. To further analyze the LDR effects at the molecular level, the genome profiling through microarray was carried out. This study will be contributed to the characterization of LDR effects in breast cancer cells and suggest the possible physiological pathways related to the LDR induced mitigated malignancy of breast cancer cells.

Deposition of ^{226}Ra in Fish Fed with Environmental Relevant Activities of ^{226}Ra

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Purpose: To determine the accumulated activity of ^{226}Ra in fathead minnow fish fed with environmental relevant dose levels of ^{226}Ra for five months in 20^{o}C water.

Methods: Fathead minnows were cultured in 20^{o}C water and were fed with fish food containing ^{226}Ra, the concentration of which was 10mBq/g, 100mBq/g, 1000mBq/g and 10000mBq/g, for about five months. Then after being sacrificed, these fish were flash frozen in liquid nitrogen and kept at -20^{o}C. Autoradiography was used to detect ^{226}Ra in fish body. In order to do that, 40μm longitudinal sections were cut at the middle of the fish body in cryostat, and each section was exposed to the solid state nuclear track detector CR-39.

Results: The greatest amount of accumulated ^{226}Ra in fish body was found for fish fed with 10000mBq/g ^{226}Ra food. The activity of ^{226}Ra deposited is about 258.0mBq/g, and the calculated dose rate is about 0.711μGy/h. For fish fed with 1000mBq/g ^{226}Ra food, the accumulated activity is about 53.9mBq/g and the dose rate is about 0.148μGy/h. For fish fed with 100mBq/g and 10mBq/g ^{226}Ra, the activity of the deposited ^{226}Ra is 20.44mBq/g and 21.84mBq/g, and the calculated dose rate is 0.056μGy/h and 0.06μGy/h respectively.

Conclusion: The result shows that there's a positive correlation between the activity of ^{226}Ra accumulated in fish body and the concentration of ^{226}Ra in fish food, but it is not a linear relationship. For fish fed with lower concentration of ^{226}Ra, the percentage of accumulated radioactivity is greater than that for fish fed with higher concentration of ^{226}Ra.