Speakers Abstracts

SYMPOSIUM I: Oxidative Stress and Aging
Sponsored by CIHR Institute of Aging

Wednesday, June 11th, 2014
8:40 AM – 11:50 AM
River Building Theatre 2200

SA01

Redox activities of flavonoids at the membrane interface: Implications for mitochondrial oxidative stress

Brian Bandy, College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon, SK S7N 5C9.

While the role of mitochondrial ROS generation as a cause of aging is enigmatic, protecting mitochondria from oxidative stress can have important implications for age-related diseases and healthspan. In experiments with yeast we have asked whether decreased mitochondrial ROS generation influences lifespan. In other experiments in vitro and in animal models we have asked whether flavonoids can decrease mitochondrial oxidative stress and apoptosis, and investigated some of the mechanisms involved. In particular our findings suggest that certain flavonoids can act at the interface of membranes, where they may bioaccumulate and cooperate with vitamins C and E. Our recent experiments suggest that the anthocyanin class of flavonoids could be especially effective as mitochondrial protectors.

SA02

Mitochondrial H_2O_2 signalling involving heme transfer between proteins

Meena Kathiresan[1], Dorival Martins[1], and Ann M English.[1]
[1]Department of Chemistry and Biochemistry, Concordia University, 7141 Sherbrooke West, Montreal, Quebec, Canada, H4B 1R6.

In exponentially growing yeast cells, cytochrome c peroxidase (Ccp1) is targeted to mitochondria where it acquires heme and accumulates in the mitochondrial intermembrane space. When the fermentable source (glucose) is depleted, cells switch to respiration and mitochondrial H_2O_2 levels rise. Given its efficiency as a H_2O_2 scavenger in vitro, it has long been assumed that CcP activity consumes and detoxifies mitochondrial H_2O_2. However, by live cell fluorescence microscopy and immunoblotting, we find that Ccp1-GFP (in cells chromosomally expressing the fusion protein) and Ccp1 begin to exit mitochondria when cells switch to respiration. Apoproteins can cross the outer mitochondrial membrane and, consistent with Ccp1's translocation as the apoprotein, we observe no extramitochondrial CcP activity. In contrast, the activity of the peroxisomal/mitochondrial catalase, Cta1, increases in respiring wild-type but not in ccp1Δ cells so we hypothesized that on oxidation by respiration-derived H_2O_2, Ccp1 transfers its heme to Cta1. In support of our hypothesis, five days after glucose depletion, we detect 4-fold higher Ccp1 protein levels in mitochondria from the cta1-null strain vs wild-type cells. Hence, in the absence of Cta1 as a mitochondrial heme

acceptor, significantly more Ccp1 remains trapped as the holoprotein in this organelle. In vitro experiments with apomyglobin as a heme acceptor confirm that H_2O_2 induces Ccp1 heme transfer and mass spectrometric analysis reveals that this involves heme labilization by oxidation of Ccp1's proximal iron ligand, His175. Altogether, our study unravels an unorthodox mode of H_2O_2 signaling that has relevance in oxidative stress and elucidates a novel regulatory mechanism of heme transfer and protein localization.

SA03

Increased DNA double strand break repair as a consequence of exposure to oxidative stress

Louise Winn, Department of Biomedical and Molecular Sciences, Queen's University.

Oxidative stress caused by exposure to xenobiotics can result in DNA damage. Specifically, we are interested in DNA double strand breaks, which can be repaired through homologous recombination and non-homologous end-joining. These DNA repair pathways are not error free and can result in detrimental genetic changes. Interestingly, histone acetylation status appears to be important during DNA repair and therefore changes in histone acetylation/deacetylation due to exposure to environmental chemicals may affect the integrity of DNA repair. This talk will focus on the use of both a transgenic animal model, and *in vitro* cell models to study the mechanisms of increased double strand break repair that is linked to exposure to a number of xenobiotics that cause toxicity through oxidative stress.

SA04

Oxidative stress in oncodynamics

Gurmit Singh, Department of Pathology and Molecular Medicine, McMaster University.

The predominant reactive oxygen species in cancer cells is hydrogen peroxide generated by the mitochondria as a necessary byproduct of oxidative phosphorylation. This oxidative stress in cancer cells results in a cascade of molecular events including up-regulation of antioxidant mechanism(s). One of the consequences is the up-regulation of an antiporter namely system Xc, which enables cancer cells to import cystine and efflux glutamate.This abnormal secretion of glutamate from tumor cells into the tumor environment causes physiological disturbance refered to as "Oncodynamics". The term Oncodynamics refers to the impact of the tumor on the body. It includes effects such as pain, depression, pain, etc. In my lecture I will discuss the novel hypothesis that oxidative stress generated in cancer cells is the cause of cancer induced-pain and cancer induced-depression.(supported by CBCF)

SA05

Testing the sulfhydration of peptides and proteins with sulphide

Artur Jarosz[1] and Bulent Mutus.[1]
[1]Department of Chemistry & Biochemistry, University of Windsor.

We will report on the results of our in vitro studies on the reaction of sulphide with a series of peptides and proteins. In these studies, the peptides and proteins were exposed to [HS-] ranging from sub- uM to mM at physiological pH. The degree of sulfhydration was assessed by a combination of UV/vis spectroscopy, MS and functional assays. (Supported By an NSERC Discovery Grant)

SA06

The role of ROS in autophagy in cancer

Spencer B. Gibson, Department of Manitoba Institute of Cell Biology, University of Mantioba.

Oxygen regulation is important in regulating normal cellular process but when deregulated leads cellular stress, this contributes to the development of various human diseases including cancers. Autophagy is one of the first lines of defense against oxidative stress where autophagosomes are created that engulf proteins and organelles to be degraded by selective lysosomal self-digestion process. This provides the cell essential elements and energy to survive cellular stress. Hence, autophagy is the survival pathway conferring stress adaptation and promoting viability. The autophagy pathway is induced and up-regulated in response to increased intracellular reactive oxygen species (ROS) or hypoxia contributing to cell survival. However, increasing evidence demonstrated that autophagy can lead to cell death under cellular stress conditions. In addition, altered autophagic signalling pathways are frequently found in many human cancers. We found that autophagy contributes to survival and cell death depending upon the length of time following cellular stress and the context to anti-cell death signals. In addition, the formation of autolysosomes provides a novel target for lysosome disrupting agents to convert a cell survival response to a cell death response mediated by ROS. This could lead to novel strategies to kill drug resistant cancer cells.

SA07

The impact of vitamin C on the premature aging disorder Werner syndrome

Michel Lebel, Department of molecular biology, medical biochemistry, and pathology, Centre de Recherche sur le Cancer de l'Universite Laval.

Werner syndrome is a premature aging disorder caused by mutations in a RecQ-like DNA helicase. Mice lacking the helicase domain of the WRN homologue exhibit many phenotypic features of WS, including a pro-oxidant status and a shorter mean life span compared to wild-type animals. Vitamin C supplementation rescued the shorter mean life span of Wrn mutant mice and reversed several age-related abnormalities in adipose tissues and liver endothelial defenestration, genomic integrity, and inflammatory status. To better assess the impact of vitamin C on the health span of such mice, we crossed Wrn mutant mice with mice that cannot synthesize their own vitamin C (like humans) and performed different physiological measurements. The double mutant mice showed a severe reduction in their life span (7 months instead of 24 months) with a minimum of vitamin C in drinking water (0.05% w/v). Although we did not detect a significant increase in reactive oxygen species in the tissues of these double mutant mice, we observed an increase in mitochondrial DNA mutation in the liver of these mice. Double mutant mice exhibit a shorter stature than wild type animals, hypogonadism, a severe decrease in bone density, earlier defenestration of liver endothelial cells than single Wrn mutant mice, and an increase of several pro-inflammatory cytokines (IL-1, IL-6, TNF-alpha, and M-CSF). Several of these phenotypes were reversed with a higher concentration of vitamin C (0.4% w/v) in drinking water. Our results indicate that vitamin C impacts directly on the pro-inflammatory status of double mutant mice.

SA08

Cancer cells exploit hypoxia-activated eIF4E2-directed protein synthesis to drive tumor progression

Jim Uniacke[1], J. Kishan Perera[2], Gabriel Lachance[2], Camille Francisco[2] and Stephen Lee.[2]
[1]Department of Molecular and Cellular Biology, University of Guelph, Guelph, Ontario;
[2]Department of Cellular and Molecular Medicine, University of Ottawa, Ottawa, Ontario.

Human tumors display considerable diversity in their genetic makeup but share common physiological attributes such as a hypoxic microenvironment that contribute to the malignant phenotype. Hypoxia induces the formation of reactive oxygen species, which stabilize hypoxia inducible factors that activate a selective transcriptional and translational hypoxia response program. Hypoxic cells switch from eukaryotic initiation factor 4E (eIF4E) to eIF4E2/HIF-2α-directed cap-dependent translation to synthesize many of their proteins. Here, we show that diverse human cancer cells exploit eIF4E2-directed protein synthesis to form cellular masses larger than approximately 0.15 mm, the diffusion limit of oxygen. Cancer cells depleted of eIF4E2 are indistinguishable from control cells under normoxic conditions, but are unable to survive and proliferate in low oxygen conditions. Activation of eIF4E2-directed translation is essential for cancer cells to form a hypoxic tumor core in in vitro spheroids and to form detectable tumors in in vivo xenograft assays. In contrast, the eIF4E-directed protein synthesis pathway alone cannot sustain cellular adaptation to hypoxia in vitro or confer tumorigenic potential in xenograft assays. These data demonstrate that the phenotypic expression of the cancer genome requires translation by the eIF4E2/HIF-2α-directed hypoxic protein synthesis machinery.

SYMPOSIUM II:
Oxidative Stress and Cardiovascular Disease

Wednesday, June 11th, 2014
3:00 PM – 6:30 PM
River Building Theatre 2200

SA09

TDAG51 as a modulator of oxidative stress in atherosclerosis

Richard Austin[1], Gazi S. Hossain[1], Edward Lynn[1], Jeffrey G. Dickhout[1], Sarka L. Hotak[1], Bernardo Trigatti[1] and Dov Shiffman Celera.[1]
[1]Department of Medicine,McMaster University.

Apoptosis caused by endoplasmic reticulum (ER) stress contributes to atherothrombosis, the underlying cause of cardiovascular disease. TDAG51, a member of the pleckstrin homology-related gene family, is induced by ER stress, causes apoptosis when overexpressed and is present in lesion-resident macrophages and endothelial cells. In this study, TDAG51-/-;Apoe-/- mice were generated to assess the causal role of TDAG51 in atherogenesis. A significant reduction in atherosclerotic lesion and necrotic lipid core size was observed in TDAG51-/-;Apoe-/- versus TDAG51+/+;Apoe-/- mice, despite any changes in lesion morphology and ER stress. TDAG51 deficiency altered several processes in macrophages and endothelial cells that increase cytoprotection against oxidative and ER stress, enhance PPARγ-dependent reverse cholesterol transport and upregulate peroxiredoxin-1, an antioxidant enzyme having anti-atherogenic properties. Two independent, case-control studies showed that genetic variants in the TDAG51 region associate with cardiovascular disease. These findings provide evidence that TDAG51 modulates multiple cellular stress pathways known to reduce atherosclerotic lesion development and progression, suggesting that regulation of TDAG51 expression may have therapeutic potential for the treatment of cardiovascular disease.

SA10

Oxidative stress and cytokines in heart failure

Pawan Singal, Institute of Cardiovascular Sciences St. Boniface Hospital Research Centre, University of Manitoba, Winnipeg, Canada.

In addition to its antitumor effects, the proinflammatory cytokine, tumor necrosis factor (TFNα), has also been shown to be cardiodepressent and responsible for various cardiovascular complications. Since clinical trials with anti-TNFα were not a success, it appears that still much needs to be learned for a full comprehension of the role of TNFα in heart biology. Another cytokine, interleukin-10 (IL-10) has been shown to have anti-inflammatory properties. It is suggested to counterbalance many adverse effects of TNFα. IL-10 suppresses the production of TNFα and many other proinflammatory cytokines. TNFα–induced oxidative stress as well as apoptosis are also known to be mitigated by IL-10. Moreover, improvement in cardiac function after treatment

with various drugs is also shown to be associated with an increase in IL-10 content. We have recently obtained evidence that IL-10 also activates the innate signaling in cardiomyocytes. Such signaling may also involve an activation of TLR4, its co-receptor CD14 and a downstream protein MyD88 in an intricate manner to control apoptosis as well as synthesis of some of the other primary cytokines. Furthermore, this effect of IL-10 is receptor mediated. Based on these data, it is suggested that an optimal balance between IL-10 and TNFα may be a new therapeutic strategy for a healthier heart. (Supported by CIHR).

SA11

Complex stabilization and destabilization of cytokines mRNA by NADPH oxidase production of O_2^-

Sheldon Magder[1] and Imad Al Gholeh.[2]
[1]Department of Critical Care Medicine, McGill University; [2]University of Pittsburg.

Expression of inflammatory cytokines is tightly regulated by transcriptional and post-transcriptional mechanisms for the activities of these molecules need to be quickly activated for efficient immune protection but also turned off to avoid an excessive response. Our objective was to examine the role of reactive oxygen species in this regulation. We previously showed that NADPH oxidase-derived superoxide (O_2^-) increases the expression and activity of inflammatory mediators in response to TNF-α and LPS. In this study, we determined if part of this increase in activity is due to stabilization by O_2^- of the mRNA of three inflammatory mediators: interleukin-8 (IL-8) interleukin-6 (IL-6) and intercellular adhesion molecule-1 (ICAM-1). TNF-α increased mRNA stability of ICAM-1, IL-8 and IL-6 by a p38 MAPK dependent mechanism, but this stabilization did not involve NADPH oxidase or O_2^-. LPS treatment alone did not alter stability of these molecules, but surprisingly the antioxidant N-acetyl cysteine (NAC), the flavine inhibitor diphenylene iodonium (DPI) and siRNA against Nox2, Nox4 and the p22phox subunit of NADPH oxidase all enhanced IL-8 mRNA stability in LPS-treated cells by a mechanism that involved ERK1/2, p38 MAPK and the mRNA destabilizing factor tristetraprolin. This indicates that O_2^- decreased stability of IL-8 mRNA induced by LPS. On the other hand, the opposite occurred with ICAM-1 and IL-6 in LPS-treated cells and IL-6 and ICAM-1 in TNF α-treated cells. NAC decreased their mRNA stability indicating that O_2^- stabilized their mRNA. In conclusion, we show that NADPH oxidase contributes to post-transcriptional mRNA stability regulation of IL-8 and propose a model for the complex underlying mechanism, which is dependent upon agonist (LPS versus TNF-α) and target molecule (IL-8 versus IL-6 and ICAM-1) and involves tristetraprolin, p38 and Erk1/2 MAPK.

SA12

Distinct roles high and low molecular weight FGF-2 in heart pathology; an overview

Elissavet Kardami[1,2,3], [1] Department of Human Anatomy and Cell Sciences, University of Manitoba, MB; [2]Department of Physiology, University of Manitoba, MB; [3]Institute of Cardiovascular Sciences, St.Boniface Res. Cntr, Winnipeg, MB.

Cardiac connective tissue cells (mainly fibroblasts and myofibroblasts) export growth factors and cytokines affecting cardiac cell survival and growth in a paracrine and autocrine fashion. Fibroblasts are major producers of the growth factor FGF-2 in rodents and humans.

FGF-2 is accumulated as high molecular weight (Hi) and low molecular weight (Lo) isoforms. Both Hi and Lo FGF-2 are secreted by cardiac fibroblasts to the extracellular space, they are present in human pericardial fluid, and they can exert distinct and often opposing activities on cardiac cells. Lo-FGF-2 has potent cytoprotective properties, acting as a pre- and post- conditioning agent; Lo-FGF-2 protects from acute drug-induced cardiotoxicity and cell death. Hi-FGF-2, on the other hand, which is upregulated by chronic cardiac disease-associated bioactive molecules such as angiotensin II and catecholamines, promotes cardiomyocyte hypertrophy, and secretion of matricellular proteins associated with fibrosis. Mitochondria-associated FGF-2 activities are also isoform-specific; exposure of isolated cardiac mitochondria to Lo-FGF-2 protects from calcium-induced permeability transition, while exposure to Hi-FGF-2 is toxic, inducing mitochondrial permeability transition. Selective targeting of FGF-2 isoforms would be required to prevent the deleterious effects of Hi-FGF-2, while preserving or potentiating the beneficial effects of Lo-FGF-2.

SA13

Differential influence of fatty acids on ischemic reperfusion injury in cardiomyocytes

Grant Pierce[1], Ganguly, R.[1], Hasanally, D.[1] and Ravandi, A.[1]

[1]Department of Physiology,University of Manitoba.

Trans fatty acids (TFAs) are generally believed to be deleterious to cardiovascular health. These data have been largely obtained from their effects on the vasculature. However, the effects of these TFAs directly on the heart have not been studied. Furthermore, the effects of trans fatty acids TFAs on the vasculature differ depending upon the type of TFA used: the industrially produced TFA elaidic acid (EA) or the ruminant TFA vaccenic acid (VA). It is possible, therefore, that the effects of TFAs on the heart may be different as well. In the present study, the effects of EA and VA on apoptotic and autophagic markers during non-ischemic, ischemic (ISCH) and ischemia/reperfusion (IR) conditions were studied. Chronic dietary interventions in mice and isolated cardiomyocytes were the models employed. Autophagic and apoptotic markers were unchanged in comparison to control in low density lipoprotein receptor deficient mice whose diets were supplemented for 16 weeks with VA or EA. Isolated rat cardiomyocytes were exposed to medium containing the fatty acids for 24 hours. VA and EA had no significant effect on biomarkers of apoptosis or cell death under control conditions. However, VA decreased the content of oxidized phospholipids content in cardiomyocytes in comparison to control and EA treated cells. Cardiomyocytes pre-treated with EA exhibited an increased sensitivity to simulated ISCH and IR as shown by an increase in cell death compared to control. This was achieved through augmented apoptosis. Conversely, VA decreased the number of dead cells during ISCH and IR. We conclude that not all TFAs are deleterious to the heart. EA predisposes cardiomyocytes to ISCH/IR injury whereas VA is cardioprotective during these conditions. This anti-apoptotic effect of VA may be due to a change in the oxidized phospholipid content in cardiomyocytes prior to ISCH.

This work was supported by a grant from CIHR and indirect support from St Boniface Hospital Foundation.

SA14

Changes in *Drosophila* mito proteome during aging and following chaperone-mediated lifespan extension

Robert M. Tanguay[1], Genevieve Morrow[1], Hyun-Ju Kim[1] , Ornella Pellerito[1], Maxime Bourrelle-Langlois[1] and Karlfried Groebe[2], Andre Schrattenholz.[2]

[1]Laboratory of Cell and Developmental Genetics, Departement de biologie moleculaire, biochimie medicale et pathologie, Institut de Biologie Integrative et des Systemes and PROTEO, Universite Laval, Quebec, Canada G1V 0A6; [2]ProteoSys AG, Carl-Zeiss-Str. 51, D-55129 Mainz, Germany.

Mitochondria can regulate lifespan through different mechanisms and their proteome is highly regulated due to their involvement in key cellular processes such as energy production and apoptosis. Thus the accumulation of post-translational modifications on major mitochondrial proteins, such as ATP synthase subunit βand VDAC, has been reported to correlate with aging in different organisms. To unveil the aging-associated changes in the mitoproteome of control and long-lived flies over-expressing Hsp22, a high-throughput 2D-PAGE analysis was performed on control flies during aging and in HSP22+ flies. In addition to highlighting the high degree of complexity of the mitoproteome and of protein post-translational modifications during aging, the results confirm the decline of mitochondrial function during aging of control flies. Moreover, the mitoproteomic profile of long-lived Hsp22 over-expressing flies during aging is distinct to the one of control flies. This result and the set of highly abundant proteins in these flies suggest that the small mitochondrial heat shock protein favor the maintenance of mitochondrial function/ homeostasis. Interestingly, the protein showing the largest changes between control and HSP22+ flies in mitoproteomic profiling is the lysozomal enzyme cathepsin D. The mitochondrial localization of cathepsin D in Drosophila was confirmed by Percoll gradient centrifugation. Cathepsin D was found in the NP-40 soluble fraction of mitochondria upon fractionation but cathepsin D was insensitive to proteinase K and trypsin even in the presence of detergents. While the exact function of cathepsin D in the mitochondria is not known, it could be involved in protein quality control either by promoting degradation of oxidatively-damaged proteins or favoring mitochondrial protein turnover. (Supported by CIHR, MiMage)

SA15

Examining the interaction between Nox5 and the AngII/AT1R pathway in podocytes

Chet E. Holterman[1], Jeffery Kopp[2], Rhian M. Touyz[3] and Christopher R.J. Kennedy.[1, 4]

[1]Kidney Research Centre, Ottawa Hospital Research Institute, Ottawa, Canada; [2]National Institutes of Health, NIDDK, Bethesda, Maryland; [3]Institute of Cardiovascular and Medical Sciences, University of Glasgow, United Kingdom; [4]Faculty of Medicine, University of Ottawa, Ottawa, Canada.

Background: Reactive oxygen species (ROS) production via NADPH oxidases (Noxs) plays a critical role in diabetic nephropathy (DN). While Nox5 is absent from the rodent genome, transgenic mice with podocyte-specific Nox5 expression develop severe pathological changes that are further exacerbated by the onset of diabetes. The Nox family is known to interact with the angiotensin 1 receptor (AT1R) and may play a role in AngII/AT1R driven renal damage. Our aim was to investigate whether the renin-

angiotensin-aldosterone-system (RAAS) is linked to Nox5 activity in our animal model.Methods: Cortical lysates from transgenic and control animals were queried for expression of RAAS pathway components. We then utilized a novel inducible transgenic mouse line expressing Nox5 in podocytes to investigate the effects of exogenous AngII on SBP and kidney function. Nox5 expression was induced prior to implantation of osmotic minipumps. Following AngII (1000ng/kg/min) or Losartan (20mg/kg) minipump implantation SBP was measured by tail cuff. Albuminuria was determined via spot urine collection and albumin ELISA at weekly intervals over the course of the experiment.Results: Nox5 transgenic animals displayed significant increases in expression of several components of the endogenous RAAS pathway including Renin, AT1R, ACE2, as well as Cox2 indicating RAAS pathway activation in response to increased oxidative stress. Delivery of AngII via osmotic mini-pump to Nox5 inducible animals increased SBP to a greater extent than for non-transgenic animals. Furthermore, albuminuria was significantly increased in Nox5-expressing mice receiving AngII. Preliminary results indicate that treatment of Nox5-expressing mice with Losartan does not prevent Nox5 induced increases in systolic blood pressure.Conclusions: The RAAS pathway has been linked to the Nox family of enzymes. Our results suggest that increased oxidative stress induced by Nox5 in podocytes leads to upregulation of the endogenous RAAS pathway. Furthermore podocyte specific expression of Nox5 renders animals more sensitive to AngII resulting in a greater increase in systolic SBP and increased albumin leakage suggesting a feedback loop between Nox5 and the RAAS pathway.

SA16

Altered mitochondrial bioenergetics and cellular redox conditions link high fat diets to the etiology of skeletal muscle insulin resistance

Christopher G.R. Perry, Department of Kinesiology and Health Science, York University, Norman Bethune College, Rm. 344, 4700 Keele St., Toronto, ON, Canada, M3J 1P3.

Insulin resistance precedes the development of Type 2 Diabetes and stems from a prolonged period of over-nutrition and physical inactivity. While the prevalence of this condition continues to increase the precise mechanisms triggering insulin resistance remain elusive. Mounting evidence implicates high consumption of dietary fat as a specific trigger of insulin resistance, particularly in peripheral tissues including skeletal muscle which comprises ~40% of body mass. A model is proposed whereby excessive lipid provision to mitochondria relative to metabolic demand increases H_2O_2 emission and oxidizes the cellular redox environment in relation to impaired insulin-stimulated glucose uptake. Central to this model is a concept of metabolic balance whereby dietary fat and physical activity alter mitochondrial oxidant emission through divergent inputs of nutrient provision and metabolic demand. An overview will be provided outlining the pharmacological and transgenic approaches used to test this model as well as emerging evidence from invasive investigations in humans.

SA17

S-Glutathionylation reactions are essential for the control of mitochondrial function

Ryan J. Mailloux, Department of Department of Biology/Institute of Biochemistry, Carleton University, 1125 Colonel By Drive, Ottawa, Ontario, K1S 5B6 Canada.

Mitochondria have a number of essential functions including provision of cellular ATP levels, cell signaling and apoptosis. These indispensable functions inherently depend on the transfer of electrons which ultimately results in the genesis of ATP and reactive oxygen species (ROS). The production of ATP and ROS are intimately linked by electron transfer from nutrients through the electron transport chain (ETC) to the terminal electron acceptor dioxygen (O_2). The production of ATP over ROS and vice versa depends on the efficiency of electron transfer and nutrient oxidation, availability of ADP, and the polarity of the mitochondrial inner membrane. Various control mechanisms converge on mitochondria to adjust ATP and ROS output in response to changing energy demands and the state of cellular redoxome. However, given that mitochondrial function is heavily dependent on redox reactions and the mitochondrial protein is rich in cysteine, it is important to consider the role of protein cysteine thiol oxidation in the control of mitochondrial function. S-glutathionylation has emerged as a key covalent modification required for control of protein function in mitochondria. Various mitochondrial proteins and enzymes involved in nutrient oxidation, amino acid metabolism, oxidative phosphorylation, antioxidative defense, solute transport, and mitochondrial dynamics have been found to be S-glutathionylated. This modification involves the formation of a disulfide bridge between a protein cysteine thiol and glutathione (GSH). Here, what is known about S-glutathionylation reactions and their impact on mitochondrial metabolism and ROS production will be examined. Specifically the role of S-gluthionylation in the control of Complex I, Complex II, Complex V, and various Krebs cycle enzymes will be discussed as well as the reversal of S-glutathionylation by glutaredoxin-2 (Grx2). Further, the implications of deregulated mitochondrial S-glutathionylation in the pathogenesis of cardiac disease, diabetes, obesity, and neurological disorders and the potential for development of mitochondria-targeted drugs that restore the S-glutathionylated proteome will also be examined.

SYMPOSIUM V:
Oxidative Stress, the Environment and Health and Nutrition (Session 1)

Thursday, June 12th, 2014
2:30 PM – 6:00 PM
River Building Theatre 2200

SA18

Bioactive dietary fibre and phenolics in flaxseed are carriers of antioxidants- an essential physiological function in oxidative stress

Bushra Madhour[1], Adrien Ondet[1], Mehri Hadi Nezhad[1] and Farah Hosseinian.[1,2]
[1]Food Science and Nutrition Program, Department of Chemistry, Carleton University, Ottawa, Ontario, Canada K1S 5B6, [2]Institute of Biochemistry, Carleton University, Ottawa, Ontario, Canada K1S 5B6.

The aim of this study is to evaluate the prebiotic capability of flaxseed dietary fiber, to enhance bacterial growth and survival, and to increase the antioxidant potential in kefir. Microbial analysis, pH, total titratable acidity (TTA), and antioxidant activity were measured weekly in the kefir model over a 28- day cold storage at 4°C, with the addition of flaxseed meal and inulin from Jerusalem artichoke, which was used as a positive control. The Kefir, supplemented with flaxseed meal, revealed a marked increase in the viability of bacteria. The highest microbial count (9.4 log cfu/ml) and acidity (0.21% TTA) was seen on day 14, which was significantly higher than the kefir control (7.9±0.61 log cfu/ml and 0.25%TTA) ($P < 0.05$). The antioxidant activity was measured using oxygen radical absorbance capacity (ORAC). The highest antioxidant activity (μmol TE /g) was seen in the kefir containing flaxseed meal (11.51±1.17) which was significantly higher than the kefir control (1.04±0.02) and inulin (4.54±0.34) ($P<0.05$). On the other hand, kefir containing meal showed 5-10% increase in minerals solubility. Crude mucilage resulted in significantly ($P<0.05$) higher total phenolic content (350±20 mg GAE/kg) and antioxidant activity (51.2±3.3 μmol TE /g) than pure mucilage. Higher antioxidant activity of crude mucilage could be demonstrated that dietary fibres in flaxseed are co-passenger of phenolics. This study has also demonstrated that fermented dietary fibre in flaxseed is a promising prebiotic model that enhances the growth of lactic acid bacteria as well as a potential antioxidant activity.

SA19

Ascorbylperoxide generated in parenteral nutrition induces oxidative stress and loss of alveoli in the lung of newborn guinea pig, a characteristic of human bronchopulmonary dysplasia

Jean-Claude Lavoie[1], Wesam Elremaly[1] and Therese Rouleau.[1]
[1]Department of Pediatrics and Nutrition, University of Montreal.

Bronchopulmonary dysplasia is a chronic lung disease affecting 30-50% of infants born before 30 weeks of gestation. Its main characteristic is a low alveolar development. Little is known on the etiology. However, oxidative stress is a key event. Parenteral nutrition received by these premature newborns is contaminated by $H_2O_2$and ascorbylperoxide. Previous data suggest that the infused ascorbylperoxide has a greater oxidative power than H_2O_2. Hypothesis: Ascorbylperoxide induces the essential oxidative stress leading to the lost of alveoli, following an oxidation of redox potential. Objective: To document in animals, the impact of ascorbylperoxide ± H2O2on oxidative stress markers and alveolarization index. Methods: Three-days old guinea pigs (n= 6-8 per group) received intravenous continuous infusion (200 mL/kg/d) of solution containing dextrose and 0, 20, 60 or 180 µM ascorbylperoxide ± 350 µMH_2O_2 (concentrations measured in parenteral nutrition). After four days, lungs were collected for determination of alveolarization index (number of intercepts between a standardized line and histological structures (nb/mm)) and redox potential of glutathione (GSH and GSSG by capillary electrophoresis). Western Blot (relative to tubuline) was used to measure protein levels of cleaved caspase-3 (apoptosis marker) and NFkB& Nrf2 in nuclear fraction (biological markers of oxidative stress). Comparisons were by factorial ANOVA (p less than 0.05).Results: Alveolarization index was lowered only by ascorbylperoxide (from 37±1 to 30±2 nb/mm). Cleaved caspase-3 was higher in animal infused with ascorbylperoxide (from 0.7±0.1 to 3.1±1.2). This effect was lower in presence of H_2O_2 (from 0.7±0.2 to 2.0±0.3). Ascorbylperoxide as well as H_2O_2 have oxidized the redox potential from -211±2 to -202±2 mV, reaching a plateau with the highest concentration of ascorbylperoxide. Nuclear levels of NFkB were increased only in ascorbylperoxide group (from 0.6±0.1 to 1.4±0.2) whereas the positive impact of ascorbylperoxide on Nrf2 (from 1.1±0.4 to 3.6±0.7) was revealed only in presence of H_2O_2. Conclusion: In lungs, the stronger oxidative power of the infused ascorbylperoxide, relatively to H_2O_2, could explain the specificity of ascorbylperoxide to induce the lost of alveolar tissue following an exaggerated apoptosis. The results suggest that redox potential is not the key element in these processes. (Funded by the Canadian Institutes for Health Research MOP-115035).

SA20

RedoxSYS™ Diagnostic Systems: The first and only clinical test to provide a complete measure of redox in a biological system

Luoxis: Alessandro Orlando, MPH Clinical Research Epidemiologist and Brian Kolasinski, Director of Commercial Development

For over the last 15 years a wide variety of research and publications have demonstrated the significant correlation between redox reactions and critical illness and injury. Historically though the challenge to applying these findings has been the inability to take a true systemic

measurement of oxidative stress. Most studies for example have defaulted to analyzing one or more individual markers, or trying to adapt a methodology not suitable for clinical use. Recognizing this shortcoming, over a decade ago our scientific team began development work on what would become the RedoxSYS™ platform. For the first time, there now exists a bench top reader that can provide a true global measurement of redox in a biological sample in the form an ORP (oxidation-reduction potential) reading.

SA21

Induction of ER stress and apoptosis by acrolein, a lipid peroxidation-derived aldehyde

Diana Averill-Bates, Département des sciences biologiques, Université du Québec à Montréal, Montréal, Québec.

Acrolein is a highly reactive α,β-unsaturated aldehyde that is a by-product of endogenous lipid peroxidation, which arises from oxidative stress. It is also a ubiquitous environmental pollutant that is generated mainly by smoke. Acrolein has been identified among the most hazardous toxicants in cigarette smoke. This aldehyde has been associated with respiratory diseases, Alzheimer's and Parkinson's, atherosclerosis, cardiovascular diseases and diabetes. Acrolein reacts with many cellular targets such as proteins and DNA, and disrupts multiple biochemical pathways. Damage to cellular proteins by acrolein could lead to the accumulation of aberrantly-folded proteins in the endoplasmic reticulum (ER) and cause ER stress. The unfolded protein response (UPR) is an adaptive mechanism that allows cells to mitigate ER stress and to restore homeostasis. This process involves three ER sensors: protein kinase RNA (PKR)–like ER resident kinase (PERK), activating transcription factor 6 (ATF6) and inositol-requiring enzyme 1 alpha (IRE1α). If homeostasis cannot be restored, the cell is eliminated by apoptosis through the ER. This study determines the mechanisms involved in acrolein-induced apoptosis mediated by the ER and possible links with the ER stress response in human A549 lung cells. The exposure of cells to acrolein induced several ER stress markers after shorter times of 15 to 30 min. Acrolein increased the expression of ER chaperone protein BiP and activated the three ER sensors: (i) the survival/rescue molecules PERK and eukaryotic initiation factor 2 alpha (eIF2α) were phosphorylated; (ii) ATF6 underwent cleavage; and (iii) IRE1α was phosphorylated. Acrolein caused apoptotic cell death mediated by the ER after 2 h, which was characterised by the induction of CHOP and activation of calpain and ER caspase-4. Calpain and caspase-7 were the initiating factors for caspase-4 activation in acrolein-induced apoptosis in A549 cells. These results increase our knowledge about cellular responses to acrolein in lung cells, which have important implications for human health. (Financial support: NSERC Canada)

SA22

Bioactive molecules in oat and their relation to oxidative stress

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Oats have been grown for thousands of years, mainly as an animal feed crop, but have gradually became an important part of the human diet. Oats are often consumed as whole grain and therefore provide the human's body with nutrients and bioactive compounds. Research has mainly been

focussed on vitamins, sterols, polyphenols, and dietary fibers. Biological activities such as antioxidants, cholesterol lowering and anti-inflammatory have been reported in vitro and in vivo. There is now also an interest in the bioactivity of peptides from hydrolyzed oat proteins. To follow suit, this study was then conducted to determine the effect of oat bran protein tryptic digests at 1, 10 and 100 mg/g HFD on oxidative stress markers (antioxidant enzymes, vitamins, nitrates, and protein carbonyls) in mice fed HFD alone and HFDs containing different concentration of the digested proteins. There was no difference in calorie intake or in body weight gain between the HFD and HFD plus protein digests groups. In liver, there was a significant increase of vitamin C levels in experimental groups (623-646µg/g wet tissue) compared to HFD (467µg/g) and chow (485µg/g) groups. No change was observed for vitamin A, C, or E in liver, brain, heart or lung. The mice that received 10 mg digested oat proteins/g HFD had lower levels of advanced oxidation protein products in heart and brain compared to levels found in these tissues in HFD and other treatment groups. Based on these data digested oat proteins have the potential to attenuate oxidative stress resulting consumption of diets high in fats.

SA23

Understanding oxidative stress in pregnancy

Sandeep Raha[1], Anna Lechowicz[1], Neetu Rakhra[1], Robyn Pereira[1], Justin Crane[1] and Alison Holloway.[2]

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With the global rise in the incidence of obesity, greater numbers of women in their reproductive years are obese during pregnancy. Obesity during pregnancy leads to a number of maternal and fetal complications including altered fetal growth and reduced fetal survival. While the mechanisms responsible for these adverse outcomes are not well delineated, oxidative stress in the placenta has been implicated. Reactive oxygen species are important signaling molecules in placental development and function. Environmental or dietary insults can trigger changes in the balance of reactive oxygen and reactive nitrogen species (i.e., oxidative and/or nitrative stress) within the placenta. These changes can compromise fetal growth and survival. Our laboratory has been focused on understanding the role of oxidative stress in dictating placental function. We have developed a rodent model of maternal obesity that exhibits many of the outcomes seen in obese women. This model exhibits increased fetal death and reduced fetal growth in association with increased levels of placental superoxide dismutase 1 (SOD1) expression and decreased mitochondrial cytochrome c oxidase activity. Furthermore there was evidence of increased oxidative damage in the placenta of the obese rats. In order to understand the role of the mitochondrial electron transport chain function (ETC) and ROS signaling in placental development, we attenuated mitochondrial ETC function using the complex III inhibitor Antimycin A in HTR8SV/Neo cells, a human first trimester trophoblast cell line. Inhibition of trophoblast ETC activity resulted in increased mitochondrial ROS production and increased trophoblast invasion. Attenuation of ROS level using N-acetyl cysteine resulted in the normalization in the levels of in vitro invasion. Interestingly, increased trophoblast invasion in our rodent model of maternal obesity was associated with poor placental oxygenation.

Therefore, oxidative stress may impact placental pathologies by affecting trophoblast function.

SA24

Teratogens induce oxidative and embryonic stress responses in the organogenesis-stage embryo

Barbara F. Hales**,** Department of Pharmacology & Therapeutics, McGill University, Montreal, QC, H3G 1Y6.

Although tremendous progress has been made in deciphering the effects of drugs or environmental exposures that disrupt normal development, the aetiology of 60-70% of all birth defects is still unknown. Our lab uses two model teratogens, hydroxyurea (HU) and 5-bromo-2'-deoxyuridine (BrdU), to investigate how the embryo responds to insult. Treatment of timed pregnant CD1 mice on gestation day 9 with either HU or BrdU results in dose-dependent increases in fetal resorptions and malformations. HU exposure induces curly tails, abnormal limbs (oligodactyly, hemimelia, and amelia), and short ribs; BrdU causes polydactyly and delayed ossification of the sternebrae and vertebrae. Both HU and BrdU alter redox homeostasis in the embryo, inducing oxidative stress and activating redox sensitive transcription factors such as the p38 and JNK mitogen-activated protein kinases (MAPKs). Reduction of oxidative stress decreases both teratogenicity and MAPK activation. In HU exposed embryos, inhibition of the p38 pathway increases fetal mortality, whereas JNK inhibition increases the incidence of hindlimb defects. Interestingly, an increase in the formation of 4-hydroxynonenal (4-HNE) protein adducts is observed in the caudal malformation-susceptible areas of HU exposed embryos; 4-HNE, a small unsaturated aldehyde, is produced by lipid peroxidation. One the proteins targeted by HU is glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Pharmacological inhibition of the nuclear translocation of GAPDH with deprenyl leads to an enhancement of malformations, specifically those of the hindlimbs, lumbosacral vertebrae, and tail, without further depleting glutathione. Microarray analyses reveal that the expression of genes involved in the MAPK pathways, DNA repair, cell cycle checkpoint activation and apoptosis are affected. Together, these data suggest that GAPDH and the MAPK pathways may serve a pivotal role as stress sensors during development. The response of the embryo to stress plays a role in determining its fate. Funded by CIHR MOP-57867.

SA25

NADPH-oxidase dependent mechanisms that determine the fate of the innate immune response to respiratory viruses

Nathalie Grandvaux[1]and Karin Fink.[1]
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Beside their reputed damaging role, Reactive Oxygen Species (ROS) have recently been characterized as modulators of signaling pathways mainly through reversible post-translational modifications of thiol residues in proteins. Viruses have long been reported to induce ROS production in the host cells, but the origin and function of these ROS has for long been ignored. Our work in progress is aimed at understanding the host innate immune response triggered by the first cells encountered by respiratory ssRNA viruses, the airway epithelial cells. This response is not only required to restrict viral replication and spreading, but also orchestrates the development of an adaptive immune response. Our studies have clearly demonstrated that ROS produced by two distinct

members of the ROS-generating NADPH oxidase enzyme family, NOX2 and DUOX2, positively contribute to the antiviral host response mounted airway epithelial cells. The signaling cascades leading to the activation of two major transcription factors, NF-κB and IRF-3, which control the expression of antiviral and proinflammatory cytokine genes are under the control of NOX2-dependent superoxides. On the other hand, DUOX2-dependent extracellular hydrogen peroxide production is induced by a novel delayed antiviral pathway and is required to sustain the Interferon-mediated antiviral response. The molecular mechanisms, and more specifically the proteins that undergo reversible oxidation during this response, are currently being investigated.

SA26

Oxidative stress and the marine environment - "radical" management

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Organisms living in the marine intertidal zone encounter extreme variations in environmental parameters that challenge their ability to acquire and utilize oxygen and/or deal with stresses that trigger oxidative damage to their cellular macromolecules. For gill-breathing intertidal organisms, the twice daily aerial exposures at low tide initiate a progression through hypoxia and into anoxia and have led to the development of excellent anoxia tolerance among organisms such as snails, clams and barnacles. Many of these also tolerate whole body freezing during low tide exposures in the winter and their anoxia tolerance is a crucial part of freezing survival. When animals are again submerged at high tide, tissue oxygenation is rapidly restored and with this comes a burst of reactive oxygen species generation as both oxygen availability and metabolic rate soar. A key element of anaerobiosis is strong metabolic rate depression to minimize energy use when oxygen-based metabolism is cut off. Against a background of strong translational and transcriptional suppression under anoxia, selected genes are up-regulated to facilitate survival and a remarkable number of these encode enzymes of antioxidant defense, potentially serving two key roles: (a) maintaining long term viability of cells during extended hypometabolism, and (b) defending against high rates of oxyradical formation during the rapid return to aerobic conditions. This talk will highlight newly recognized mechanisms of metabolic arrest with a focus on the strategies used to suppress energy-expensive cell functions (e.g. transcription, translation), implement and regulate stress responsive gene expression, and upgrade cellular antioxidant defense mechanisms. In the marine snail, *Littorina littorea*, this involves up-regulation of mainline antioxidant enzymes, metal-binding proteins (ferritin, metallothionein) and chaperone proteins under anoxia as well as coordinated mechanisms of metabolic arrest that include attention to phosphorylation-mediated suppression of metabolic enzymes, transcription and translation, as well as microRNA inhibition of mRNA translation. These mechanisms have many lessons to teach us about biochemical adaptation in extreme aquatic environments as well as helping to define the underlying strategies for oxygen/oxidative stress management across phylogeny. For more information please visit www.carleton.ca/~kbstorey. Funded by NSERC.

SYMPOSIUM VI: Oxidative Stress and Neurodegeneration
Sponsored by CIHR Institute of Neurosciences, Mental Health and Addiction

Friday, June 13th, 2014
8:30 AM – 11:00 AM
River Building Theatre 2200

SA27

Anti-psychotic drug induced oxidative stress involves translocation of apoptosis inducing factor

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Antipsychotic drugs are the primary therapeutic agents used for the treatment of schizophrenia and other mental illnesses. While these drugs are effective in treating the symptoms of the mental illnesses they are also associated with the development of a variety of adverse side effects. For example, the typical antipsychotic drug, haloperidol, is associated with the development of early onset Parkinson's-like symptoms and late onset tardive dyskinesia. The atypical antipsychotic drugs, such as olanzapine, are associated with the development of metabolic disorders, such as type 2 diabetes, and cardiovascular problems. Prolonged treatment with haloperidol results in the development of tardive dyskinesia in 20-40% of the treated patients. Tardive dyskinesia is potentially irrerversible and has been associated with a poor quality of life. The mechanisms involved in the development of tardive dyskinesia are not currently understood and the treatments remain unsatsfactory. Previous studies from our lab have reported that free radicals are produced in the striatum during haloperidol treatment. Additionally we have demonstrated the potential role for a free radical scavenger and anti-apoptotic molecule in mitigating haloperidol induced tardive dyskinesia-like symptoms, the vacuous chewing movement in rats. These investigations further implicated increased oxidative stress in the development of tardive dyskinesia. The present study was aimed at investigating the role of Apoptosis Inducing Factor (AIF) in the haloperidol mediated adverse effects. Translocation of AIF from the mitochondria to nucleus promotes cell death independantly of the caspase cascade. To examine how AIF may contribute to haloperidol induced apoptosis, AIF translocation was observed in three separate experimental paradigms. Dopamine D2 receptor transfected SHSY5Y cells were incubated with Haloperidol were assessed AIF translocation using immunohistochemistry. Similarly, AIF immunofouresence was characterized in striatal tissues from rats administered haloperidol for 28 days. Finally, human striatal tissues from patients who had received haloperidol were assessed for AIF immunoflouresence. There was increased nuclear AIF immunoflouresence observed in cells, rat and human striatal tissues. These results provide novel evidence implicating the involvement of AIF in haloperidol-associated apoptosis and its relevance to the development of antipsychotic drug related adverse effects such as early onset Parkinson's disease and late onset tardive dsykinesia.(supported by NSERC, Canada)

SA28

The role of oxidative stress in the pathogenesis of hepatic encephalopathy

Christopher Rose, Department of Medicine
CRCHUM, Universite de Montreal.

Hepatic encephalopathy is a neuropsychiatric disorder; a major complication of liver disease. Impairment in liver function leads to a reduced capacity to clear ammonia (via urea cycle) and subsequently hyperammonemia arises. Consequent neurotoxic levels of ammonia are considered to play a major role in the pathogenesis of hepatic encephalopathy. However, a correlation between ammonia and severity of neurological impairment is poor. Oxidative stress is another factor believed to play a role in the pathogenesis of this syndrome as it has been shown to exacerbate the neuropsychological effects of hyperammonemia. In the setting of liver disease, oxidative stress represents a systemic phenomenon induced by several mechanisms: decreased antioxidant synthesis, increased systemic release of oxidant enzymes, generation of reactive oxygen species and impaired neutrophil function. Furthermore, it has been demonstrated that high ammonia concentrations can induce oxidative stress. However, in the setting of chronic liver disease, the observed significantly lower degrees of hyperammonemia (less than 500 μM) do not induce cerebral nor systemic oxidative stress; defining these 2 pathogenic factors as independent. Data from both animal and human studies sustain that there is a synergistic effect between systemic oxidative stress and ammonia in the pathogenesis of hepatic encephalopathy and induction of cerebral oxidative stress may be associated with severe neurological symptoms.

SA29

Embryonic and fetal reactive oxygen species formation, oxidative DNA damage and repair and nuclear factor-E2-related factor 2 (Nrf2) in teratogenesis and postnatal neurodevelopmental deficits

Peter G. Wells[1], Lutfiya Miller[1], Annmarie Ramkissoon[2] and Aaron Shapiro.[2]
[1]Faculty of Pharmacy and Dept. of Pharmacology & Toxicology, University of Toronto;
[2]Faculty of Pharmacy, University of Toronto.

Birth defects and postnatal abnormalities in brain function may be caused by enhanced embryonic and fetal levels of reactive oxygen species (ROS) due to endogenous sources or in utero exposure to drugs and environmental chemicals. We have used several model teratogens (phenytoin, methamphetamine, methanol, ethanol, thalidomide and hydrolysis products, methylmercury and ionizing radiation), together with outbred and genetically modified mouse models in embryo culture and in vivo to elucidate embryonic and fetal pathways of ROS formation and detoxification, and DNA repair, which together may regulate conceptal ROS levels and macromolecular damage, and thereby constitute important biochemical determinants of risk. ROS formation may result from embryonic prostaglandin H synthase (PHS)-catalyzed bioactivation of teratogens, or via activation/induction of NADPH oxidases. The embryopathic nature of ROS in teratogenesis is suggested by reduced DNA oxidation and embryopathies following pretreatment with exogenous PEG-catalase or the free radical spin trapping agent phenylbutylnitrone. The particular pathogenic role of physiological levels of ROS-initiated oxidative damage to DNA, as distinct from oxidation of fetal proteins and lipids, and from ROS-mediated signal transduction, is

revealed by increased postnatal neurodevelopmental deficits in untreated knockout progeny deficient in the DNA repair protein oxoguanine glycosylase 1 (OGG1). OGG1 knockout progeny are similarly more susceptible than wild-type littermates to fetal brain DNA oxidation and neurodevelopmental deficits caused by in utero ethanol exposure at a relatively low dose that does not cause structural birth defects. Similarly in embryo culture, conditional knockout embryos deficient in the breast cancer 1 protein (BRCA1), which regulates several DNA repair pathways including those for oxidative lesions, exhibit enhanced DNA oxidation and embryopathies caused by ethanol and methamphetamine compared to wild-type littermates, corroborating the pathogenic importance of oxidative DNA lesions and revealing an important developmental role for BRCA1 in protecting the embryo from oxidative stress. Numerous protective antioxidative proteins and DNA repair proteins like OGG1 are upregulated by nuclear factor-E2-related factor 2 (Nrf2) in response to oxidative stress, and this upregulation and protection against neurodevelopmental deficits caused by in utero exposure to methamphetamine were lost in Nrf2 knockout progeny, indicating a developmentally important role for Nrf2. (Support: CIHR)

SA30

Mechanisms of natural products antioxidants: a case study with garlic-derived organosulfur compounds

Derek A. Pratt, Department of Chemistry, University of Ottawa, Ottawa, Canada.

For three decades, enormous effort has been directed to identify and characterize the reactivity of natural product antioxidants in an attempt to understand the epidemiological evidence that links diet and the incidence of degenerative disease. This effort is confounded by the reality that 'antioxidants' can have one of several modes of action, such as radical-scavengers (chain-breaking antioxidants), peroxide decomposers (preventive antioxidants) or electrophiles (indirect antioxidants).1 Radical scavengers have historically received the most attention due to the 'free radical theory' of aging and age-related disease, however, most of them are neither sufficiently reactive nor bioavailable to account for their biological activity. Herein, we will present our work towards our understanding of the biological activity of garlic, the world's oldest medicine.2 The unique organosulfur compounds found in garlic and related medicinal plants from the Allium genus can, in principle, react by trapping radicals, decomposing peroxides and reacting as electrophiles. We will discuss our efforts to identify which activity is responsible for garlic's medicinal properties, with an emphasis on the unique radical chemistry uncovered in doing so.3-9

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D. A. Chem. Commun. 2013, 49, 8181. (9) Zheng, F.; Li, B.; Pratt, D. A. 2014, submitted.

SA31

***Lymnaea stagnalis*: a platform for system-wide investigations of neuronal aging and age-associated memory impairment – lipid peroxidation and PLA2 activation as instruments of age-associated memory impairment**

Willem Wildering[1], Jon R. Lee[1], Shawn Watson[1]and Petra Hermann.[1]
[1]Departmet of Biological Sciences, University of Calgary.

The pond snail *Lymnaea stagnalis* has a long track record as a neurobiological model system. Lymnaea's compact central nervous system (CNS) with its many large and in many cases individually identifiable neurons has been attractive to many neuroscientists interested in relating molecular and electrical properties of neurons and neuronal circuits to behavior. Behavioral and physiologic control functions of many of Lymnaea's identified neurons and the circuits in which they partake have been characterized. Together, these features provide unique opportunities for system-wide, molecule to whole animal behavior investigations of fundamental questions in neurobiology, including aging and age-associated decline in nervous system functions. In recent years, our laboratory has shown that aging Lymnaea develops selective transcription-dependent long-term memory (LTM) failure in two different associative learning paradigms. We have also linked this age-associated memory impairment (AIM) to a decline in excitability of key interneurons instrumental in LTM formation in each of these two paradigms. Both behavioral and electrophysiological symptoms of old age can be reproduced in young mature animals through induction of oxidative stress by means of injection of hydrophilic free radical generator AAPH. Remarkably, both experimentally induced and normal age-associated behavioral and electrophysiological deficits were reversed by inhibition of phospholipase A2 (PLA2). Together our recent data puts lipid peroxidation and PLA2 at the fulcrum of age-, oxidative stress- and inflammation-associated neurophysiological and behavioral impairment in this model system. Intriguingly, our results reiterate growing evidence implicating similar processes as an important aspect of the etiology of mammalian (and human) age-associated deficiencies of the brain, including perhaps mild cognitive disorder (MCI) and Alzheimer's disease.

SA32

NO-problem, Oxidative stress in a stem cell model of Parkinson's Disease

Scott Ryan, Department of Molecular and Cellular Biology, University of Guelph.

Neurodegeneration in Parkinson's Disease (PD) is associated with both aberrant mitochondrial function as well as impaired proteostasis in dopaminergic neurons (DA) of the substantia nigra pars compacta. A strong association has been reported between PD and exposure to mitochondrial toxins such as the environmental pesticides paraquat, maneb, and rotenone. These toxins are associated with increased oxidative stress that may link mitochondrial dysfunction with aberrant proteostasis. Using a robust, patient-derived human induced pluripotent stem cell model (hiPSC) of PD that allows for comparison of A53T-SNCA mutant cells against isogenic mutation-corrected controls, we generated DA neurons (hNs). An analysis of mitochondrial function via in these neurons

identified perturbations in mitochondrial respiration specific to A53T mutant hNs. A decrease in maximal respiratory capacity was observed in A53T hNs coupled to an increase in production of reactive oxygen (O_2^-) and nitrogen species (NO). Furthermore, we report a novel molecular pathway whereby basal as well as toxin-induced oxidative stress inhibited the MEF2C-PGC1α transcription network in A53T hNs, leading to neuronal death. This occurred, in part, through redox-based modification of MEF2C that prevented the transcription factor from binding to the PGC1α promoter. Our data provide mechanistic insight into gene by environment interactions in the pathogenesis of PD and identify NO as a critical stressor in the disease.

SYMPOSIUM VII:
Oxidative Stress, the Environment and Health and Nutrition (Session 2)

Friday, June 13th, 2014
1:00 PM – 4:50 PM
River Building Theatre 2200

SA33

The transcription factor Nrf3 (NFE2L3): role in detoxification and cancer

Volker Blank[1,2], Gregory Chevillard[1,2], Zaynab Nouhi[1,2], Anna Derjuga[1,2], Meenakshi B. Kannan[1,2], Isadore Dodard-Friedman[1,2] and Marilene Paquet.[2]
[1]Department of Department of Medicine, Lady Davis Institute for Medical Research; [2]McGill University.

Cap 'n' Collar (CNC) basic leucine zipper transcription factors are crucial regulators of mammalian gene expression, stress response and tumorigenesis. The CNC protein Nrf3 (NFE2L3) forms heterodimers with small Maf proteins and binds to MARE (Maf recognition element) or ARE (Antioxidant response element) consensus sequences. Our cellular studies revealed that Nrf3 exists in three forms, located in the ER, cytoplasm and nucleus. We found that Nrf3 has a high turnover and we have evidence that the protein is controlled through the ubiquitin proteasome pathway. We showed that Nrf3-/- mice develop and grow normally under non-challenging conditions. We found that knockout mice express reduced levels of the detoxification enzyme NAD(P)H quinone oxidoreductase (NQO1). In order to examine whether Nrf3 is involved in chemical-induced carcinogenesis, we challenged the mice with benzo[a]pyrene (B[a]P), a common carcinogen found in cigarette smoke and charbroiled food. Our studies revealed that Nrf3 null mice are highly susceptible to B[a]P exhibiting significantly higher morbidity and mortality than their wild type counterparts. Pathology analysis of affected tissue sections revealed a high incidence of T-cell lymphoblastic lymphoma in B[a]P-treated Nrf3 null mice when compared to wild type animals. Lymphoblastic lymphoma occasionally metastasized into the lung as demonstrated by perivascular lymphocytic infiltration. We conclude that absence of Nrf3 predisposes mice to lymphoma, suggesting a protective role of this transcription factor in hematopoietic malignancies. Together, our studies demonstrate that Nrf3 is a stringently regulated transcription factor at the cellular level, and that it plays a role in the control of detoxification genes and in the response to carcinogens *in vivo*.

SA34

Impact of air pollutant exposure on oxidative stress and endothelial dysfunction

Prem Kumarathasan[1], Renaud Vincent[1], Erica Blais[1], Anushuyadevi Saravanamuthu[1], Agnieszka Bielecki[1], Ballari Mukherjee[1], Stephen Bjarnason[1], Josée Guénette[1] and Patrick Goegan[1].
[1]Environmental Health Science and Research Bureau, Environmental and Radiation Health Sciences Directorate, HECSB, Health Canada, Ottawa, ON.

While exposure to ambient air contaminants is clearly associated with adverse health outcomes, disentangling mechanisms of pollutant interactions remains a challenge.We aimed to characterize changes in markers of free radical reactions and cardiovascular health in rats after inhalation of urban particulate matter, ozone, ormixtures of particles plus ozone.Fischer 344 rats were exposed for 4h to ozone (0, 0.4, 0.8 ppm) and EHC-93 particles (0, 5, 50 mg/m^3) either individually or as mixtures. Bronchoalveolar lavage fluid (BALF), BAL cells, blood and plasma were analysed for various biomarkers of effect immediately and 24h post-exposure.Inhalation of ozone increased lipid oxidation products (pless than0.05) in BAL cells immediately after exposure, and increased (p less than0.05) total protein, neutrophils and mature macrophages in the BALF 24h post-exposure. Ozone exposure resulted in increased reactive oxygen species in the BALF (Ozone main effect, p less than 0.05), while formation of reactive nitrogen species (RNS) as indicated by 3-nitrotyrosine levels correlated with dose of urban particles (EHC-93 main effect or EHC-93 x Ozone interactions, p less than 0.05). Carboxyhemoglobin levels in blood exhibited particle exposure-specific changes (p less than0.05). In plasma, ROS and RNS products were increased (p less than 0.05) after inhalation of particles; the effect on 3-nitrotyrosine was abrogated after exposure to ozone plus particles (EHC-93 x Ozone, p less than 0.05). The vasoactive peptides BET-1 and ET-1 were increased in plasma after inhalation of particles alone or ozone alone, but the effects were attenuated by co-exposure to the contaminants (EHC-93 x Ozone, p less than 0.05). Circulating levels of endothelins were positively correlated with oxidative stress. Our results indicate that pollutant-specific changes can be amplified or abrogated following multi-pollutant exposures. Nitrative stress and endothelinergic imbalance emerge as potential key pathways of air pollutant health effects, notably of ambient particulate matter.

SA35

Novel interventions in the resuscitation of asphyxiated neonates: combating oxidative stress

Po-Yin Cheung[1,2]
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Introduction: Neonatal asphyxia is a major cause of death and morbidity in the survivors. Cardiopulmonary resuscitation is commonly required for asphyxiated neonates. Using swine models of neonatal asphyxia, we examined novel interventions in the resuscitation of asphyxiated neonates so that the ischemic-hypoxic and reperfusion-reoxygenation injuries could be alleviated as well as cardiovascular supportive therapies during the recovery after asphyxia. Materials and Methods: Term newborn piglets (1-3 day-old, 1.5-2.5kg) were anesthetized and

instrumented for continuous systemic and regional hemodynamic monitoring and intermittent blood sampling. Both acute non-survival and chronic survival swine models were used. Hemodynamic parameters including cardiac output, systemic and pulmonary arterial pressures, common carotid, superior mesenteric and renal arterial flows were recorded. Hypoxic and oxidative stress injury markers were studied in plasma and tissues.Supplemental oxygen,anti-oxidant (N-acetylcysteine), mitochondrial metabolic modulator (cyclosporine) and matrix metalloproteinases inhibitor (doxycycline)were studied in a blinded, block-randomized fashion in acutely and chronically instrumented piglets subjected to hypoxia/asphyxia-reoxygenation (n=8-10 per group). **Results:**Swine models of neonatal asphyxia were established. Cardiogenic shock (40-50% of normoxic baseline), hypotension (30-35 mmHg) and severe metabolic acidosis (pH 7.01-7.15) occurred in hypoxic/asphyxiated-reoxygenated piglets which subsequently developed myocardial stun (60-70% of normoxic baseline)with reduced regional blood flows during recovery. Oxygen (18%, 21%, 50% and 100%) used in resuscitation caused a dose-dependent oxidative injury in the recovery of systemic and regional perfusion in acutely (6 hours) and chronically (5 days) instrumented piglets. Further studies also demonstrated the protective effects with the use ofN-acetylcysteine, cyclosporine and doxycycline, which were administered 5-10 minutes after reoxygenation. Associated reduction in tissue oxidative stress was observed in treated piglets compared to controls. Conclusions: Using swine models of neonatal asphyxia, 21% oxygen could be used effectively in neonatal resuscitation with reduced oxidative stress in tissues (Neonatal Resuscitation Guidelines 2010). Novel resuscitative interventions are developed and cardiovascular supportive therapies are better understood. The findings are important for translation into clinical scenarios.

SA36

Proteomic changes in response to arylamine free radical formation in HL-60 cells

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In this study, we have investigated the changes to the cellular proteome resulting from the intracellular formation of arylamine free radical metabolites. HL-60 cells contain significant amounts of the hemoprotein, myeloperoxidase (MPO), which, similar to other peroxidase enzymes, catalyzes one-electron oxidation of arylamines using H_2O_2 as a cofactor. We have previously shown that arylamine metabolism by MPO results in the formation of arylamine free radical metabolites which correlated with protein radical formation (possibly due to protein oxidation by the arylamine metabolites). Conditions were optimized for arylamine-induced protein radical formation with minimal cytotoxicity and these conditions were used to carry out proteomic studies. We utilized p-aminoglutethimide (AG) as a representative arylamine. We identified 43 proteins that were significantly changed upon AG metabolism of which 18 were up-regulated and 25 were down-regulated which were associated with apoptosis, proteolysis, and oxidative stress pathways. We focused on further experiments that dealt with the

apoptotic response in order to corroborate the proteomic findings. The quantitative proteomic data showed that AG peroxidative metabolism led to the down-regulation of critical anti-apoptotic proteins responsible for inhibiting the release of pro-apoptotic factors from the mitochondria as well as cytoskeletal proteins such as nuclear lamina. The pro-apoptotic response was confirmed with flow cytometry which demonstrated apoptosis to be the main mode of cell death, and this was attenuated by MPO inhibition. This response correlated with the intensity of AG-induced protein radical formation in HL-60 cells, which may play a role in cell death signaling mechanisms. Apoptotic pathways were created using manual curation as well as an automated method; there were some similarities and differences. To our knowledge, this is the first study to characterize the proteomic changes that occur resulting from arylamine free radicals and to implicate protein radical formation as an associated observation.

SA37
Oxidative stress in sepsis: necessary or injurious?

Alison Fox-Robichaud , Division of Critical Care, Thrombosis and Atherosclerosis Research Institute, McMaster University, DBRI C5-106, 237 Barton St East, Hamilton, Ontario, L2L 2X2.

Sepsis is the systemic response to infection. This results in an increase in oxidative stress, believed to be essential for pathogen killing, but also occurs as a result of result of reduced organ perfusion and may be responsible for direct organ injury. There is some evidence to suggest that restoring certain antioxidants are depleted in patients who develop sepsis and may need to be replaced, however this has not been demonstrated in a recent large trial. It is also unclear what happens in situations when the oxidant/antioxidant balance is disturbed in conditions such as obese patients who become septic. We hypothesized that organ dysfunction would worsen in a murine model of diet-induced obesity with sepsis. In this presentation I will provide an overview of the complex relationship between oxidants and antioxidants in sepsis. Then I will share some early data about the role of oxidative stress in our obese sepsis model.

SA38
Glutathione-dependent metabolism of xenobiotics

David Josephy, Department of Molecular & Cellular Biology, University of Guelph, Guelph, ON.

Glutathione is a central player in the cell's defences against both oxidative stress and reactive electrophiles. We have been studying several aspects of the biochemistry of glutathione. Bacterial mutagenicity assay strains have been constructed, expressing human GST T1-1 – both the wild-type enzyme and variants encoded by single-nucleotide polymorphic (SNP) alleles. GST T1-1 catalyzes the glutathione-dependent activation of ethylene dibromide to a mutagenic intermediate. These expression systems have been used to evaluate the structural and functional consequences of GST T1-1 SNPs. We are also studying the enzymes of the mercapturic acid pathway, which catalyze the metabolism of xenobiotics to N-acetylcysteine conjugates. Glutathione transferase (GST) enzymes catalyze the conjugation of glutathione with reactive functional groups of endogenous compound! s and xenobiotics, including halonitroaromatics. We have characterized dinitronaphthalene derivatives as inhibitors of human GST enzymes (Alpha, Mu, and Pi

classes). The most potent inhibition was observed towards GSTs M1-1 and M2-2; IC50 values for 1-methoxy- and 1-ethoxy-2,4-dinitronaphthalene were in the nanomolar range. Inhibition accompanies the formation, at the enzyme active site, of very stable Meisenheimer complex intermediates. We are also examining the specificity of NAT8, the microsomal cysteine conjugate N-acetyltransferase that catalyzes the final step of mercapturic acid formation, for aromatic cysteine-conjugate substrates. These investigations will help to clarify the biological roles of glutathione-dependent toxication and detoxication processes.

SA39

Iron and complementary feeding of breast-fed infants

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Rationale: The American Pediatric Society and Health Canada both recommend that iron fortified cereal (most iron unabsorbed) or meat (most iron absorbed) be introduced to all breast-fed infants at six months of age to support iron stores and linear growth. Evidence suggests that the iron contained in these foods, particularly cereal, is not fully absorbed and will collect in the colon with the possibility of free radical generation and intestinal inflammation. Objectives: To assess the safety of the recommended first solid food for exclusively breast-fed infants from a free radical perspective.

Design and methods: Ninety exclusively breastfed infants are being randomized to 1 of 3 feeding groups: iron fortified cereal (FeCer), iron fortified cereal with fruit (FeAOXCer) or meat (M). Urine and stool samples are collected before introduction of study foods (4-5.5 months) and 3 weeks after introduction of these foods to assess the following markers: urinary F2 isoprostanes (LC-MS-MS), urinary 8OH-deoxy guanosine (ELISA), fecal calprotectin (ELISA), reactive oxygen species (ROS-HPLC) and non-heme iron generation (Colorimetric) in the stool, and the fecal microbiome by 16S RNA gene pyrosequencing. Results: preliminary results are presented for isoprostanes and ROS generation: urinary isoprostanes (base: 10.1+/_7.2; after feeds 12.8 +/_ 7.6 ng/mg/ml creatinine; n=25) did not differ over time or between feeding groups at either baseline or after feeds. ROS did not differ between groups at either sampling time nor within the FeAOXCer and M group over time, however there was a trend to increased free radical generation in the FeCer group (base: 0.018 + 0.019; after feeds 0.037 + 0.015 RU; n=12; P = 0.087). Conclusion: Our hypothesis (1) was that iron fortified cereals would provide unabsorbed iron that would reduce the ability of the colon to resist oxidative stress. As well, infants receiving iron-fortified cereals would be more likely to experience systemic oxidation as reflected in urinary isoprostanes 92). Hypothesis 1 has been supported from this preliminary data on a subset of the total sample. This suggests that the newborn breast-fed infant may not cope with large amounts of unabsorbed iron in the colon.

SA40

Redox balance and non-alcoholic fatty liver disease and non-alcoholic steatohepatitis.

Xiaolei Jin, Toxicology Research Division, Bureau of Chemical Safety, Food Directorate, Health Canada.

Liver is a metabolic active organ responsible for many vital functions. The worldwide increasing prevalence of obesity makes non-alcoholic fatty liver disease (NAFLD) the most common chronic liver disease. NAFLD is a hepatic manifestation of metabolic disorder with triglyceride accumulating in the liver in the absence of excessive alcohol consumption, which may progress to non-alcoholic steatohepatitis (NASH) involving in inflammation and apoptosis. Molecular mechanisms leading to the development and progression of NAFLD are not fully understood. However, recent findings suggest elevated free fatty acids (FFA), endoplasmic reticulum (ER) stress, hepatic iron deposition, mitochondrial dysfunction, dysregulation of fatty acid uptake, and triglyceride synthesis and secretion, hepatic insulin resistance, and altered cholesterol and lipoprotein metabolism are potential contributors to NAFLD, most of which, if not all, are influenced by redox balance. Free radicals generated from multiple sources may affect molecular states leading to activation/deactivation of enzymes, transcription factors, and nuclear receptors, and alterations in signaling cascades, intracellular trafficking, and posttranslational modifications of proteins. Evidence suggests that NAFLD is associated with altered antioxidant defense, and modulation of redox state using antioxidant therapy may be effective treatment for this disease.

SA41

Tyrosine nitration in membranes: role of lipid-derived radicals and modulatory action of tocopherols

Silvina Bartesaghi[1,2] and Rafael Radi[1,2] , [1]Departamento de Bioquímica and [2]Center for Free Radical and Biomedical Research, Facultad de Medicina, Universidad de la República, Montevideo, Uruguay.

Protein tyrosine nitration is a postranslational modification mediated by nitric oxide-derived oxidants. The nitration mechanisms in hydrophobic biostructures (i.e. biomembranes and lipoproteins) differ from those in aqueous solution mainly because of the high concentration of unsaturated fatty acids present in these compartments, the exclusion of antioxidants that are normally present in aqueous phases (e.g. glutathione) and the diffusion of species such as •NO and •NO2 which may favor nitration reactions. Indeed, many proteins shown to be nitrated either in vivo or in vitro are associated to membranes and lipoproteins. We recently proposed that lipid peroxyl (LOO•) radicals oxidize tyrosine residues to tyrosyl radicals (Tyr•): LOO • + TyrH → LOOH + Tyr • k ~ 5 x 103 M-1s-1

These reactions are relevant during lipid and protein oxidation processes in membranes and lipoproteins. However, the mechanistic and structural basis remains largely unknown. In this work, we have performed experimental and computational studies in a model system consisting of transmembrane peptides (Y4, Y8 and Y12 from the amino terminus) incorporated to phosphatidylcholine (PC) liposomes of varied fatty acid composition. Tyrosine nitration by different oxidants (e.g. peroxynitrite) was evidenced after HPLC separation and MS/UV-VIS analysis. Oxidation yields increased as a function of oxygen concentration (e.g. 10-210 µM), in parallel with an increase in lipid peroxidation products. Conversely, the presence of alpha- or gamma-tocopherol decreased tyrosine nitration and lipid oxidation. In egg yolk PC liposomes, tyrosine oxidation was larger in Y8 compared to Y4 and Y12. Molecular dynamics

simulations in a 1-palmitoyl, 2-linoleyl phospathidylcholine model membrane-containing peptides support that the probability of finding the Tyr phenolic O close to the linoleyl reactive C9 and C13 atoms (where the peroxyl radicals are located) is maximum for Y8. Kinetic and thermodynamic evidence support the LOO• reaction with tyrosine in hydrophobic milieu. Thus, the data provide mechanistic and structural insights on how lipid peroxidation-derived LOO• can oxidize tyrosine residues embedded in lipid bilayers over other competing redox processes.

SA42

Toxicogenomics analysis of the potent carcinogen dibenzo[def,p]chrysene (DBC) provides mechanistic and quantitative insights into its immunotoxicity

Nikolai Chepelev[1], Alexandra S. Long[1], Andrew Williams[1], Byron Kuo[1], Dean A. Kennedy[1], Volker M. Arlt[2],Paul A. White[1], and Carole L. Yauk.[1]

[1]Environmental and Radiation Health Sciences Directorate, HECSB, Health Canada;[2]Analytical and Environmental Sciences Division, MRC-PHE Centre for Environment and Health, King's College London.

Dibenzo[def,p]chrysene (DBC) is the most carcinogenic polycyclic aromatic hydrocarbon (PAH) examined to date. Pilot experiments revealed that acute exposure of adult male Muta™Mouse to DBC by oral gavage leads to spleen atrophy within three days of exposure. We applied toxicogenomics to evaluate the mechanisms underlying this immunotoxicity and to evaluate the utility of toxicogenomics data for human health risk assessment of DBC. Animals were exposed to 0, 2.0, 6.2, and 20.0 mg/kg bw DBC for three days by oral gavage. Genotoxicity (DBC-DNA adduct frequency, 7,8-Dihydro-8-oxo-2'-deoxyguanosine (8-oxodG) levels and micronucleus frequency), reticulocyte cytotoxicity, and global gene expression in the spleen (RNA-seq and microarrays) were measured 4-, 24-, or 72-h after the last treatment. Specific gene expression changes were confirmed by quantitative RT-PCR. Adduct and micronucleus frequencies increased in a dose-dependent fashion and were significantly above controls at all doses. All DBC doses exhibited significant blood cell cytotoxicity, measured as a decrease in the percent of reticulocytes recovered. In contrast, 8-oxodG levels were increased in the spleen at the high dose only. Toxicogenomics showed that DBC activates TP53, affects "cellular growth and proliferation, hematological system development and function, and hematopoiesis", which was evident even at the low dose. No obvious induction of genes related to oxidative stress response was detected, suggesting that oxidative stress is a secondary effect of DBC. Analysis by RT-PCR revealed that the expression of a group of genes related to apoptosis (e.g. Bbc3, Birc5, Bnip3l, Prc1) and iron metabolism (Hmox1, Fech) was inversely correlated with expression during benzo(a)pyrene-mediated spleen enlargement. Similarly, DBC expression profiles showed highly significant, inverse correlations with profiles from mice with enlarged spleens reported by other group. Dose-response modelling revealed similar departures from background levels for spleen weight and pathway gene expression changes, suggesting a point of departure of 0.095 mg and 0.21 mg DBC/kg bw per day using traditional and toxicogenomics approaches, respectively. Overall, this study provides rich mechanistic insight into immunotoxicity caused by DBC exposure and demonstrates the potential utility of toxicogenomics in defining the doses at which adverse effects occur for health risk assessment of PAHs and other potential immunotoxicants in general.

Graduate Student Symposium Abstracts
SYMPOSIUM III: Graduate Student Trainees

Thursday, June 12, 2014
8:30 – 9:45 AM
River Building Theatre 2200

GS01
Lipid peroxidation-linked mitochondrial facets of neuronal aging in an invertebrate model of normal aging
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The gradual failure of neurons in senescence is thought to involve the accumulation of free radical damage – known as oxidative stress – to cellular components over the lifetime of an organism. Under the guise of plasma membrane lipid peroxidation, oxidative stress seems to directly contribute to declining neuron excitability in aging Lymnaea stagnalis. We have recently shown that in this model system activation of a plasma membrane peroxidation repair enzyme, phospholipase A2 (PLA2), is tightly coupled to this neuronal failure, and its inhibition can restore the excitability of aged or young-oxidized neurons. Though the role of lipid peroxidation in neuronal excitability decline is now being elucidated, the root cause of this oxidative process is still unknown. It remains to be seen whether the deleterious effects of age-associated oxidative stress are initially caused by an overwhelming accumulation of free radicals overtime, or by a gradual failure in a neurons ability to provide for defensive reducing agents in the form of antioxidants. Recent studies using flavin and nicotinamide (FAD and NADH) auto-fluorescence as markers of mitochondrial redox state and metabolism suggests that neurophysiological and behavioral exponents of normal aging in our model system are paralleled by shifts in the way aging neurons handle pro-oxidant challenges. NADH and FAD redox states differ in young and old Lymnaea brains, suggesting intrinsic age-dependent differences in mitochondrial metabolic state. We also show that through PLA2 inhibition, old neurons can be induced to perform metabolically like their young counterparts. Young neurons exposed to experimental oxidative stress exhibit a metabolic fluorescence profile not unlike that of old, and this phenotype can again be reversed by PLA2 inhibition. As in the case of age-related neuronal excitability decline (previous study), we provide evidence that in Lymnaea, metabolic discrepancies between young and old are largely mediated by plasma membrane lipid peroxidation and PLA2 activity.

GS02
Protection by ascorbate and catechin against myocardial ischemia-reperfusion injury in an isolated rat heart model
Ahmed Abou Hadeed[1], Paul Lee[1]and Brian Bandy.[1]
[1]Department of College of Pharmacy and Nutrition, University of Saskatchewan.

Myocardial ischemia-reperfusion (I/R) injury occurs in situations such as angioplasty and cardiac surgeries which in severe cases can increase the risk of heart morbidities. Flavonoids such as catechins in teas, and ascorbate (vitamin C) which exists extensively in fruits and vegetables are known for their antioxidant activity and may be of benefit in protecting from I/R injury. This ex-vivo study had two objectives: the first was to determine the extent to which ascorbate or catechin alone at physiologically relevant levels can protect myocardial tissue in a

situation of I/R injury, and the second was to evaluate the possible synergistic protective effect of ascorbate and catechin when given together. Isolated rat hearts (n=48) were perfused in the retrograde mode with modified Krebs-Henseleit buffer; following stabilization and the induction of 30 min global ischemia, ascorbate (150 μM) and catechin (5 μM) were added directly into the perfusate during 90 min reperfusion. The presence of infarction and histopathological features (edema and necrosis) were evaluated with, triphenyltetrazolium chloride (TTC) and hematoxylin and eosin (H&E) stains, and indicators of apoptosis (caspase-3 activity), lipid peroxidation (thiobarbituric acid reactive substances and total malondialdehyde), and glutathione redox status were measured. The experiments showed that at these levels, ascorbate protected the heart against lipid peroxidation and cell apoptosis by 100%, while catechin protected by 67% and 90% respectively. The presence of edema and necrosis were substantially decreased by ascorbate and partly by catechin. Due to the strong protective effects of ascorbate alone, the results did not show any synergistic protective effect when ascorbate and catechin were used together. Our study is the first to report the protection by catechin at this physiological level under conditions of myocardial ischemia-reperfusion injury.

GS03

Free radical trapping agents as adjunct therapy to antipsychotic drugs for the treatment of schizophrenia

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Antipsychotic medications produce a host of adverse side effects and in patients with schizophrenia have lead to considerably high drug relapse rates and poor treatment outcomes. Adverse side effects include acute dystonic reactions, pseudoparkinsonism, tardive dyskinesia or akathisia. The molecular mechanisms related to the pathophysiology of these extrapyramidal symptoms remain unclear. However, recent studies suggest enhanced free radicals and behavioural supersensitivity collectively contribute to their pathophysiology. The aim of this study was to examine whether the free radical trapping agent, α-phenyl-N-tert-butylnitrone (PBN), can prevent the development of abnormal oro-facial movements (an animal model of tardive dyskinesia), behavioural supersensitivity and various markers of oxidative stress. Male Sprague Dawley rats were treated chronically with the typical antipsychotic drug, Haloperidol, in conjunction with PBN or its vehicle for 4 weeks. Vacuous chewing movements, lipid peroxidation (assessed with thiobarbituric acid reactive substances assay), antioxidant enzyme activities (superoxide dismutase, catalase, glutathione), locomotion and behavioural stereotypies were measured. The free radical trapping agent, PBN, prevented the development of vacuous chewing movements, indicators of behavioural supersensitivity following dopamine agonist challenge, as well as preventing lipid peroxidation and the reduction of antioxidant enzyme activities. In conclusion, these findings suggest the involvement of striatal free radicals in the development of antipsychotic induced extrapyramidal side effects. Moreover, free radical trapping agents, such as PBN, are promising prospects for the adjunct therapy of schizophrenia and the reduction of antipsychotic induced extrapyramidal side effects.

GS04

Enzyme-specific inhibition of recombinant human glutathione transferases by naphthalene analogues of 1-chloro-2,4-dinitrobenzene

Hilary Groom[1], Dr. P. David Josephy[1] , Dr. Moses Lee[2] and Pravin Patil.[2]
[1]Department of Molecular & Cellular Biology, University of Guelph; [2]Natural and Applied Sciences Division, Department of Chemistry, Hope College, Holland MI.

Glutathione transferase (GST) superfamily enzymes initiate the detoxication of many xenobiotics and byproducts of intermediary metabolism. GSTs catalyze the conjugation of glutathione with reactive functional groups of xenobiotics. Enzyme-specific GST inhibitors may have therapeutic potential, e.g., to counteract the resistance of tumour cells to chemotherapeutic agents that are detoxified by glutathione conjugation. 1-Chloro-2,4-dinitrobenzene (CDNB) is one of the most commonly used substrates for GST activity assays. We have studied the interactions of naphthalene analogues of CDNB with recombinant human GST enzymes (Alpha, Mu, and Pi classes) expressed in *E. coli*. Several dinitronaphthalene derivatives were found to be GST inhibitors. The most potent inhibition was observed towards GST M2-2; IC50 values for 1-methoxy- and 1-ethoxy-2,4-dinitronaphthalene were below 1 µM. The dinitronaphthalene derivatives react with glutathione in the enzyme active site, forming coloured Meisenheimer complex intermediates. Genotoxicity tests (Salmonella Ames assays) showed these dinitronaphthalene derivatives to be potently mutagenic. Development of therapeutically useful inhibitors based on our observations will require capitalizing on the Meisenheimer-complex chemistry while avoiding the liability of the nitroaromatic functionality, which is generally associated with mutagenicity. (Supported by the Natural Sciences and Engineering Research Council of Canada.)

GS05

Possible role for superoxide dismutase in phenylbutazone cytotoxicity in HT-29 colorectal cancer cells

Naif Aljuhani, Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, AB, Canada.

Purpose: To investigate if the carbonate radical that is generated through superoxide dismutase (SOD)-peroxidase activity would enhance phenylbutazone cytotoxicity. Methods: A cell-free system utilized UV-Vis spectrophotometry to determine if SOD would enhance the oxidation of phenylbutazone via generating carbonate radicals through its peroxidase activity which utilizes bicarbonate (HCO_3^-) and hydrogen peroxide (H_2O_2). Also, we used EPR to detect the effects of phenylbutazone on the EPR spectrum that was associated with carbonate radical. We utilized colorectal cancer cells (HT-29) to evaluate the cytotoxicity of phenylbutazone using the alamarBlue assay. Results: The oxidation of phenylbutazone was enhanced by the peroxidase activity of SOD. UV-Vis measurement showed that λmax for phenylbutazone (λ=260 nm) showed a decline in intensity. SOD-omitted reactions produced less oxidation. The cytotoxicity of phenylbutazone was significantly enhanced in the presence of H_2O_2. Using HT-29 cell lysate, H_2O_2 and HCO_3^-, we detect a putative phenybutazone radical metabolite EPR spectrum, however, this spectrum was attenuated with HT-29 cell lysate treated with diethyldithiocarbamate (SOD inhibitor). Conclusion: Phenylbutazone appears to react with carbonate radical generated through SOD peroxidase activity. Furthermore, SOD appears to play a role in phenylbutazone cytotoxicity through a cooxidation reaction that involves the carbonate radical. These findings may represent a novel mechanism of phenylbutazone-induced toxicity in HT-29 cells.

Postdoctoral Symposium Abstracts
SYMPOSIUM IV: Postdoctoral Fellow Trainees

Thursday, June 12, 2014
10:15 – 11:45 AM
River Building Theatre 2200

PDA01
Differential role of toll-like receptors in the elicitation of cardiac innate response to IL-10
Ashim Bagchi[1,2], Gauri Akolkar[1,2] , Xiaoling Gao[3], Xi Yang[4] and Pawan K. Singal.[1,2]
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Cardioprotective role of IL-10 in TNF-α-induced oxidative stress has been established very well. Recently, we reported that this protective effect may involve innate signaling via the activation of patterns recognition receptors, such as Toll-like receptor 4 (TLR4). We have also demonstrated that myeloid differentiation gene factor 88 (MyD88) plays a key role in this innate response to IL-10. In the present study, we further characterized downstream details in IL-10 stimulation of innate signaling using IL-10 knockout (KO) mice. A significant increase in the expression of TLR2 was noticed in the KO mice hearts whereas TLR4 expression was not different from the wildtype (WT). There was a significant downregulation in MyD88 expression. The expression of interleukin-1 receptor associated kinases -1 (IRAK-1) was slightly but significantly less in IL-10 KO hearts with almost no difference in IRAK-4 expression. On the contrary, IRAK-M (an inhibitor of MyD88-dependent TLR4 signaling pathway) and IRAK-2 levels were higher in the KO mice compared to WT. Levels of TNF-α in IL-10 KO mice were higher both in the heart and blood. TLR2- mediated TNF-α activation in IL-10 KO mice led to an increase in apoptosis as evident from increased expression of pro-apoptotic protein, Bax and Bax/Bcl-xL ratio. In the KO hearts, the increased proteolytic enzyme activity of caspase3/7 as well as fibrosis in the heart suggested that TLR2-mediated IRAK-M/IRAK-2 activation might enhance TGF-β and TNF-α. Thus an increase apoptosis as well as fibrosis in the IL-10 KO mice suggest that this cytokine is an important player in restoring cardiac cells from damage. (Supported by the Canadian Institutes of Health Research)

PDA02
FGF-2 isoforms and doxorubicin-induced cardiac dysfunction
Navid Koleini[1,2], Jon Jon Santiago[1,2], Barbara E. Nickel[1,2],Wattamon Srisakuldee[1,2], Davinder Jassal[1,3] and Elissavet Kardami.[1,4]
[1]Institute of Cardiovascular Sciences, St. Boniface Research Centre; [2]Department of Physiology, University of Manitoba; [3]Department of Internal Medicine, University of Manitoba; [4]Departments of Physiology and Human Anatomy and Cell Sciences, University of Manitoba.

Doxorubicin (DOX) is an effective anti-cancer drug but its use is limited by acute and chronic cardiotoxicity, mediated, at least in part, by increased oxidative stress. Strategies for myocardial protection from DOX are required. FGF-2 is a cardioprotective growth factor, produced in tissues as high molecular weight (Hi, >20 kDa) and low molecular weight (Lo, 18 kDa) isoforms. Lo-FGF-2 is known to exert acute as well as chronic beneficial effects, reducing ischemia-reperfusion injury and preventing myocardial infarction-induced tissue loss and dysfunction. The role of >20 kDa high molecular weight FGF-2 is not known. We asked if Lo-FGF-2 and/or Hi-FGF-2 could protect from

DOX-induced cardiotoxicity, using transgenic mice. In addition, studies were initiated aimed at examining FGF-2-isoform-specific effects on cell survival-associated signals. DOX was administered in wild type mice (WT), and in mice lacking all FGF-2 isoforms (K01), expressing only Hi-FGF-2 (K02), or only Lo-FGF-2 (K03). Amongst male groups, mortality and loss of contractile function (Fractional Shortening, Ejection Fraction, Endocardial Velocity) were significantly lower in the K03 mice compared to the other groups following DOX treatment, correlating with elevated levels of the channel and hemichannel protein Connexin43, the cardioprotective protein kinase C (PKC) epsilon and also of Connexin43 phosphorylated at PKC epsilon target sites. Another cardioprotective kinase, AKT, was also elevated in K03 mice. Finally, pilot studies indicated potential FGF-2-isoform-dependent differences in autophagy markers, and that K03 mouse hearts had significantly elevated levels of GAPDH. In conclusion, expression of the Lo-FGF-2 isoform is protective against DOX-induced contractile dysfunction and DOX-induced mortality, by a mechanism likely mediated by PKCepsilon, Connexin43 phosphorylation at specific sites, AKT, and a shift to glycolytic metabolism. Current studies are addressing the role of FGF-2 isoforms in preventing DOX-induced dysregulation of autophagy.

PDA03

A comparison of transient transfection methods to enhance eNOS expression in human endothelial progenitor cells (EPCs)

Maria Florian[1], Duncan Stewart[1, 2] and David Courtman.[1, 2]

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Human mononuclear cells (hMNC) processed by a surface selective cell manufacturing protocol and modified in cell culture, have been termed early outgrowth EPCs and possess a regenerative potential in cardiovascular and pulmonary vascular diseases. In our gene-enhanced cell therapy product we overexpress endothelial nitric oxide synthase (eNOS), the enzyme responsible for nitric oxide production as over-expression of eNOS has been proven to enhance the regenerative capacity of EPCs isolated from patients with cardiac ischemia injury. In the pre-clinical experimental phase, we aimed to compare different ways to transfect EPCs. The level and duration of eNOS expression was correlated to the viability of transfected cells with and without the use of the ROCK inhibitor and enhancer Fasudil. Human peripheral blood monocytes (MNCs) were obtained by leukapheresis. Monocytes were enriched by processing with an automated Sepax2 procedure. The cell product was cultured on human fibronectin coated flasks in supplemented Endothelial Basal Medium-2 (EBM-2) containing 20% human serum. Early EPCs were transfected on day 5-7 with pVAX-eNOS plasmid by one of the transfection protocols that uses cationic polymer transfection reagents: linearized polyethylenimine Jet-PEI® Macrophage and Jet-Prime®, and by electroporation protocols supplied by Maxcyte or by Gene Pulser Xcell system. The immunoblots suggest that the eNOS expression in JetPei transfected EPCs is stronger in cell cultures seeded at low density compared to high, $1.5x10^6$ vs $2.5x10^6$ cell/ml. pVAX- empty DNA also elicited the eNOS expression in first 24 hours after transfection, this effect was reduced with time, however in EPCs transfected with eNOS-DNA plasmid lasted through 48 hours. Untreated EPCs cultured in unconditioned media yielded $7.6x10^6$ cells, treatment with Fasudil increased the cell count to $12.0x10^6$ c/T175 flask. Fasudil however did not rescue the viability of JetPei transfected EPCs, the cell count in empty pVAX was $4.6x10^6$ cell vs.$4.1x10^6$ cells and that of eNOS-DNA $5.3x10^6$ vs 5.1 $x10^6$ cells unstimulated vs Fasudil-exposed EPCs respectively. MaxCyte showed robust eNOS expression in electroporated EPCs however the viability of cells was 42.4±5.1% in EP controls, 31.1±6.1 or 28.6±6.1% in eNOS transfected EPCs with or withot DNASe respectively. JetPei provides solid transfection of EPCs. Although the eNOS

expression by electroporation is robust, the viability of cells needs to be improved.

PDA04

Systemic oxidative stress induction leads to brain edema in hyperammonemic portacaval-shunted rats

Cristina R. Bosoi[1], Melanie Tramblay[1] and Christopher F. Rose.[1]
[1]Department of Hepato-neuro Laboratory, CRCHUM, Montreal University.

Background: Liver failure/disease leads to hyperammonemia, a central component in the pathogenesis of hepatic encephalopathy. There is increasing evidence oxidative stress may exacerbate the neuropsychological effects of hyperammonemia in patients with liver disease. With new highly sensitive imaging techniques, brain edema represents a common entity in cirrhotic patients with hepatic encephalopathy. As portacaval shunted hyperammonemic rats (PCA) do not develop oxidative stress or brain edema, we aimed to investigate the role of oxidative stress in the pathogenesis of brain edema in PCA rats. Methods: Oxidative stress was induced following glutathione (GSH) depletion by diethyl maleate (DEM). PCA and SHAM-operated rats received DEM (1 mg/kg/day intraperitoneally) for 10 days starting at day 18 after surgery. Rats were sacrificed at day 28 and oxidative stress markers, glutathione (GSH), malon-dialdehyde (MDA) and 4-hydroxy-2-nonenal (HNE) were assessed in arterial plasma and brain (frontal cortex) tissue. Plasma ammonia and liver function markers (AST, ALT, bilirubin) were also evaluated. Brain water content was measured using a specific gravimetric technique. Results: DEM induced a significant decrease in plasmatic GSH which lead to an increase in arterial MDA (2.5 fold) and HNE (1.4 fold) levels in PCA rats compared to non-treated PCA rats. In the brain, oxidative stress markers measured in the frontal cortex did not differ between the two groups. DEM treatment did not affect the degree of hyperammonemia or lead to an alteration in liver function in comparison to non-treated PCA rats. An increase in brain water content was observed in DEM-treated PCA rats vs non-treated PCA rats (PCA+DEM: 78.45 ± 0.13% vs PCA: 77.38 ± 0.11%, $p < 0.001$). Conclusions: DEM induced systemic, not central, oxidative stress in PCA rats. This, imposed on hyperammonemia, resulted in an increase in brain water content. Oxidative stress and brain edema were not detected in non-treated hyperammonemic PCA rats and therefore our findings suggest a synergistic effect between hyperammonemia and systemic oxidative stress is implicated in the pathogenesis of brain edema in hepatic encephalopathy.

PDA05

Effects of caloric and non-caloric soft drink intake on consumption of nutrients and lipoperoxidation in rats fed the cafeteria diet

Martine Hagen[1,2], Vanessa Giacomeli[1], Rutiane Thoen[1], Rafael Fernandes[1], Jeferson Goularte[1], Gilberto Sanvitto[1] and Adriane Bello-Klein.[1]
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[2]Hospital de Clinicas de Porto Alegre.

Obesity is an important risk factor in the development of various metabolic disorders.This study aimed to analyze the effect of caloric and non-caloric soft drink intake on consumption of nutrients, body weight gain, visceral adipose tissue, serum triglycerides anderythrocytes lipoperoxidation in rats fed cafeteria diet ad libitumover a 12 week period. Wistar rats were divided into six groups: 1) Control (Con): standard chow and water; 2)Cafeteria Diet (CD): standard chow, cafeteria diet and water; 3) Caloric Soft Drink (CS): standard chow, water and caloric soft drink; 4) Non-Caloric Soft Drink (NCS): standard chow, water and non-caloric soft drink; 5) Caloric Soft Drink and Cafeteria Diet (CSCD): standard chow, cafeteria diet, water and caloric soft drink; 6)Non-Caloric Soft Drink and Cafeteria Diet (NCSCD): standard chow, cafeteria diet, water and non-caloric soft drink. The daily average carbohydrate intake was higher in CS as compared to the Con. The caloric soft drink intake contributed to a higher (80%) consumption of simple carbohydrates in the CS as compared to the Con.

The CS consumed less protein (32%) and lipid (33%), when compared to the NCS and Con.The cafeteria diet resulted in an increase in body weight gain in CD (60%) when compared to Con and in CSCD(52%) as compared to Con and CS. The weight of visceral adipose tissue was significantly increased in CD (240%) as compared to Con; in CSCD (213%) as compared to CS; and in NCSCD (84%) as compared to NCS. The cafeteria diet intake resulted in an increase in triglyceride in CD (95%) as compared to Con, and in CSCD (70%) as compared to CS. Lipoperoxidation was increased in CD (74%), CSCD (36%), and NCSCD (14%) as compared to Con, CS, and NCS, respectively. Conclusion: Cafeteria diet resulted in an increase in body weight gain, visceral adipose tissue, triglycerides and lipoperoxidation which are related with obesity comorbidities. This study suggests an adoption of an antioxidant supplemented diet as well as a healthy life style to attenuate such comorbidities.

PDA06

Skeletal Muscle Mitochondrial Respiration and ROS production is Increased in Obese Diet Sensitive Compared to Obese Diet Resistant Women

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Differences in skeletal muscle oxidative capacity and mitochondrial function may contribute to weight loss variability in humans. We previously showed that obese diet sensitive (ODS) have a higher proportion of oxidative fibers and mitochondrial proton leak in quadriceps muscle compared to obese diet resistant (ODR) women. It was hypothesized that muscle from ODS would demonstrate higher rates of mitochondrial respiration and reactive oxygen species (ROS) production and oxidative stress prior to and following a high fat meal (HFM).Diet adherent women who completed the Ottawa Hospital Weight Management Program and demonstrated the highest (ODS) and the lowest (ODR) rates of weight loss participated in this study. V. lateralis biopsies were obtained from ODS (n=10, 52±2yrs; 93.2±5.9 kg; 35.6±1.7 kg/m^2) and ODR (n = 10, 48±2yrs; 100.9±7.3kg; 37.1±2.0kg/m^2) before and 6 h post a HFM (35% of daily caloric requirements; 60% kcal from fat, 50% saturated fat). Resting and postprandial (PP) metabolic rates assessed with indirect calorimetry were not different between ODS and ODR. PP fatty acid (FA) oxidation rates increased in a comparable manner in both groups. Mitochondrial metabolism was assessed in permeabilized muscle fibers with high resolution respirometry. FA supported respiration (1.5mM malate (M), 200 μM octanoyl carnitine (OC), 5mM ADP) was increased in ODS vs. ODR prior to (ODR vs. ODS; 9.8 ± 1.3 vs. 12.7 ± 1.6 pmol/s/mg wet wt, P less than 0.05) and 6 h post (ODR. vs. ODS; 13.4 ± 1.9 vs. 18.7 ± 2.1 pmol/s/mg wet wt, P less than 0.05; group effect p=0.08) a HFM. OXPHOS (with M, OC, 2mM pyruvate, 10mM glutamate, 10mM succinate, 10mM ADP) was significantly increased in ODS vs. ODR prior to (ODR vs. ODS; 53.9 ± 3.0 vs. 66.9 ± 4.2 pmol/s/mg wet wt) and 6 h post (ODR vs. ODS; 58.3 ± 5.1 vs. 75.3 ± 6.5 pmol/s/mg wet wt; P less than 0.05) a HFM. Mitochondrial content was not different between ODS and ODR. Hydrogen peroxide production was higher in ODS compared to ODR, pre and post HFM. This research demonstrates that muscle mitochondrial function and ROS production are greater in ODS than ODR individuals at rest and in response to HFM and this is independent of mitochondrial content.

Poster Presentation Abstracts
Poster Session I

Wednesday, June 11, 2014
11:50 AM – 2:00 PM
River Building Atrium

PPA01
Triticale bran alkylresorcinols enhance resistance to oxidative stress in obesity-induced mice

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Triticale (× *Triticosecale* Whitm.) is a cereal grain with high levels of alkyresorcinols (AR) which are concentrated in the bran. These phenolic lipids have been shown to reduce or inhibit triglyceride accumulation and protect against oxidation, however, their biological effects have yet to be evaluated *invivo*. The objective of this study was to determine the effects of ARs extracted from triticale bran (TB) added to a high a fat-diet on the development of obesity and oxidative stress. CF-1 mice were fed a standard low fat (LF) diet, 60 % high-fat diet (HF) and HF diets containing either 0.5% AR extract (HF-AR), 10% TB (HF-TB) or 0.5% vitamin E (HF-VE). Energy intake, weight gain, glucose tolerance,fasting blood glucose (FBG) levels and body composition were determined.Oxygen radical absorbance capacity (ORAC), superoxide dismutase (SOD) activity, and glutathione (GSH) assays were performed on mice liver and heart tissues.Liver and heart tissues of HF with AR extract diet groups possessed significantly higher ($P< 0.05$) peroxyl radical scavenging activity (0.53 & 0.54 µM TE/mg of protein), glutathione levels (0.68 & 0.41 µM GSH/mg of protein) and lower oxidized glutathione/reduced glutathione (GSSG/GSH) ratios (0.14 & 0.18) than HF diet groups (0.31 & 0.33 µM TE/mg of protein, 0.47 and 0.20 µM GSH/mg of protein, 0.41 & 0.51 GSSG/GSH ratios). Although other parameters did not show significant differences, mice supplemented ARs did exhibit improved FBG levels, glucose tolerance, as well as increased SOD activity in the liver in comparison to control HF mice. Findings of this study suggest that ARs may serve as a preventative measure against risks of oxidative damage associated with high-fat diets and obesity through their application as functional foods and neutraceuticals. Future studies aim to identify the *invivo* mechanisms of action of ARs and the individual homologs involved in their favourable biological effects.

PPA02
Evaluation of Flutamide-Induced Hepatotoxicity in an In Vitro Oxidative Stress-Inflammation Model

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Flutamide (FLU) is a competitive antagonist of the androgen receptor which has been used in association with castration in the treatment of metastatic prostatic carcinoma. FLU was reported to induce severe liver injury in some patients. The most common liver pathology was hepatic necrosis and cholestasis. Several experimental models suggested that an episode of inflammation during drug treatment predisposes animals to tissue injury. Inflammation caused by infections or endotoxins markedly activates NADPH oxidase. In the phagosome, superoxide radicals spontaneously

form hydrogen peroxide (H_2O_2) and other reactive oxygen species (ROS). The potential molecular cytotoxic mechanisms of FLU towards isolated male Sprague Dawley rat hepatocytes were investigated in this study using "Accelerated Cytotoxicity Mechanism Screening" (ACMS) techniques. Incubation of isolated hepatocytes for 2 hr with 75 µM flutamide induced an approximate 50% loss in hepatocyte viability (LC50, according to ACMS). A significant increase in FLU-induced cytotoxicity and ROS formation was observed when glutathione (GSH) depleted hepatocytes were used and this toxicity was decreased by the addition of N-acetylcysteine (a GSH precursor). Catalase inactivation also increased FLU-induced cytotoxicity, while the direct addition of catalase to the hepatocytes delayed cytotoxicity, suggesting that H_2O_2 generated by FLU caused the cytotoxicity. When a non-toxic H_2O_2 generating system (glucose/glucose oxidase) with peroxidase or Fe(II) (to simulate in vivo inflammation) were added to the hepatocytes prior to the addition of FLU, an increase in FLU cytotoxicity, ROS formation, and lipid peroxidation (LPO) were observed that were reversed by 6-N-propyl-2-thiouracil (a peroxidase inhibitor) or desferoxamine (an iron chelator), respectively. Potent antioxidants, resveratrol (a polyphenolic compound), and trolox (the water-soluble vitamin E analogue) significantly decreased FLU-induced cytotoxicity, ROS and LPO formation, and increased % mitochondrial membrane potential (MMP). TEMPOL (4-hydroxy-2,2,6,6-tetramethylpiperidin-1-oxyl), a known ROS scavenger and superoxide dismutase mimic, and DPPD (N,N'-diphenyl-1,4-phenylenediamine) also reversed toxicity caused by FLU. These results raise the possibility that the presence or absence of inflammation may be another susceptibility factor for drug-induced hepatotoxicity.

PPA03

The role of Prenyl Diphosphate Synthase subunit 1 in cellular bioenergetics and its implications in obesity

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The incidence of obesity is on the rise globally, and treatment options available to obese patients are limited to behavioural and dietary modifications, a very limited repertoire of medications and bariatric surgery. We have studied patients at the Ottawa Hospital Weight Management Clinic and their responses to a 900kCal meal-replacement program (OptifastTM, Nestle) and have investigated variability in rate of weight loss in highly adherent patients. When patients with the highest and lowest quintiles for weight loss were selected and compared by genome-wide association studies, prenyl diphosphate synthase subunit 1 (PDSS1) was identified as a gene locus bearing significance to rate of weight loss. Previous studies have also correlated expression levels of PDSS1 with rate of weight loss following bariatric surgery. Given its established role as the rate-limiting enzyme in the biosynthesis of Coenzyme Q10 (CoQ10), it could also modulate mitochondrial redox and reactive oxygen species (ROS) production. Therefore, the goal of this project is to ascertain the link between PDSS1, cellular energy expenditure mechanisms, and the production of ROS in the context of obesity and rate of weight loss. To do this, in vitro gene knockdown studies were conducted in a mouse myoblast cell line (C2C12). Findings to date include reduced cellular energy expenditure following PDSS1 knockdown, consistent with the idea of its implication in CoQ10 synthesis and control of cellular respiration. More specifically, basal and maximal oxygen consumption rates were

decreased by approximately 30%. In addition, state 4o (proton leak) respiration was doubled. Future directions include supplementation of growth media with CoQ10 to reverse these bioenergetic defects. Generally, we expect that findings will yield an improved understanding of the role of energy expenditure mechanisms in the development and treatment of obesity and associated conditions.

PPA04

The effects of antioxidant catalase-SKL in a co-morbid rat model of stroke and Alzheimer's disease

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The hallmark of Alzheimer's disease (AD) has been considered to be the formation of amyloid plaques from deposition of beta-amyloid protein (Aβ) in the brain, but more recently it is believed that pathologic neuroinflammation, oxidative stress and neuronal death contribute to early cognitive decline before the presence of amyloid plaques. Furthermore, all of these cellular pathologies are coincident in stroke, which has been shown to further exacerbate AD pathology. This study aims to investigate the role of oxidative stress and neuroinflammation in an aged animal model of stroke and AD treated with a genetically engineered form of the antioxidant enzyme catalase (CAT-SKL). Bilateral intracerebroventricular injections of Aβ25-35 (AD model) and/or unilateral right striatal injections of endothelin-1 (stroke model) were performed on 6-month-old male Wistar rats randomly assigned to receive intraperitoneal injections of saline or CAT-SKL (1mg/kg). The asymmetrical forelimb use task and modified sticky tape task were completed to assess motor function and the Morris water maze (MWM) was used to assess spatial reference learning and memory. The analysis of neuroinflammation and infarct volume was completed through immunohistochemical staining. Co-morbid rats demonstrated prolonged gross motor deficits in comparison to endothelin-1 alone. CAT-SKL managed to delay the appearance of gross motor deficits in the co-morbid rat, but was unable to fully prevent motor deficit. Aβ25-35 and co-morbid rats showed similar cognitive decline in the MWM with CAT-SKL preventing cognitive deficits in Aβ25-35 animals, but having no preventative effect in the co-morbid rats. This data supports CAT-SKL as a potential therapeutic to prevent mild cognitive impairment due to Aβ25-35, but treatment of co-morbid animals needs further investigation to determine whether different doses of CAT-SKL or combination with anti-inflammatory therapeutics will be effective.

PPA05

***In utero* effect of single dose of ethanol on epigenetic modifications of histone H3 in bains of fetal DNA repair-deficient Oxoguanine Glycosylase 1 (*ogg1*) knockout mice**

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In utero exposure to alcohol (ethanol,EtOH) causes structural and postnatal neurobehavioral abnormalities, termed Fetal Alcohol Spectrum Disorders (FASD). We previously reported that DNA repair-deficient oxoguanine glycosylase 1 (*ogg1*) knockout fetuses exposed to a single maternal intraperitoneal (i.p.) EtOH dose (2 g/kg) on gestational day (GD) 17 exhibited enhanced DNA oxidation and neurobehavioral abnormalities compared to wild-type littermates, implicating reactive oxygen species (ROS)-initiated DNA damage in the pathogenic mechanism. We are currently evaluating the epigenetic effects of fetal EtOH exposure on histones and DNA in*ogg1*knockout mice. In preliminary studies,

pregnant*ogg1*mice were treated on GD 17 with EtOH (2 g/kg i.p.). Fetal brains were extracted 1 hr post treatment, and acetylation of histone H3 at lysine 9 (AcH3lys9), and di-methylation of histone H3 at lysine 9 (Me2H3lys9), were assessed by western blotting. Initial results suggest a greater than 30% increase in EtOH-initiated AcH3lys9 in*ogg1*heterozygotes (+/-) compared to*ogg1*wild-type (+/+) littermates (p value less than 0.5), and further analyses are ongoing. The apparent effect of fetal*ogg1*genotype on EtOH-initiated AcH3lys9 formation suggests that the enhanced susceptibility of OGG1-deficient progeny to postnatal neurodevelopmental deficits caused by*in utero*EtOH exposure may at least in part involve oxidative DNA lesions leading to epigenetic changes in fetal brain.Funding:Canadian Institutes of Health Research.

PPA06

Low birth weight is associated with adiposity, weight loss resistance and alterations in skeletal muscle energetics and H_2O_2 production

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Maternal undernutrition is associated with increased risk for type 2 diabetes and obesity in the adult lives of offspring. Given the central role of skeletal muscle in whole body metabolism, we hypothesize that predisposition to metabolic disease is, in part, due to low oxidative capacity and dysfunctional mitochondrial energetics in skeletal muscle. We used an experimental mouse model system of maternal undernutrition during late pregnancy to examine female offspring from undernourished dams (U) and control offspring from ad libitum fed dams (C). U had reduced birth weight, rapid catch up growth, increased adiposity and impaired glucose tolerance compared to C, confirming previous studies. Weight gain and food intake post-weaning were similar for both groups. At 10 weeks of age, mice were 40% calorie restricted for 4 weeks. U lost half as much weight (15%) as controls. This is of interest based on our previous studies of weight loss variation in highly diet-adherent obese women in an intensively supervised behavioral weight loss program at the OttawaHospital. Muscle mitochondria from U had decreased coupled (state 3) and uncoupled (state 4) respiration but increased maximal respiration compared to C. Mixed fiber type muscle from U had decreased mitochondrial content and decreased leak respiration (adenylate free conditions), fatty acid oxidative capacity, and state 3 respiratory capacity through complex I in permeabilized fiber preparations. Maximal oxidative phosphorylation capacity in fibers did not differ between U and C but was significantly decreased with calorie restriction. Interestingly, similar to differences in oxygen consumption, H_2O_2 production was decreased in mixed muscle from U during leak respiration, fatty acid fueled respiration, and complex I driven respiration compared to C but was not different between groups during maximal respiration. Findings suggest that undernutrition in utero programs adaptations in skeletal muscle that are associated with altered metabolism and weight loss resistance. Funding: CIHR (INMD) to MEH and NSERC CGS to BB.

PPA07

Effect of Hsp22 over-expression on mitochondrial homeostasis

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Aging results from combination of genetic and environmental factors, which are responsible for the loss of proteome homeostasis that leads to the impairment of cellular processes. Mitochondria play an important role in the aging process due to the production of reactive oxygen species (ROS) as by-products of the electron transport chain (ETC). These ROS are highly reactive and can impair protein function. Mitochondrial proteins are particularly prone to oxidative damage due to their close proximity with ROS. Heat shock proteins (Hsps) have a protective role against protein damages by promoting their refolding and/or turnover. Drosophila melanogaster Hsp22 is localized in the mitochondrial matrix and increases lifespan and resistance to oxidative stress upon over-expression. To analyze further the effect of Hsp22 over-expression in mitochondria, biochemical studies and a high-throughput 2D-PAGE analysis were performed. Hsp22 is rapidly induced by paraquat (oxidative stress generator) in young flies demonstrating a clear link between oxidative stress and this sHsp. To investigate the impact of Hsp22 on the age-induced accumulation of oxidative damages to mitochondrial proteins, 2D-oxyblots were performed on control and long-lived flies over-expressing Hsp22. The mitoproteins from Hsp22 over-expressing flies showed less oxidative damage suggesting a diminution in ROS production or a better protein protection/turnover. Accordingly, mitochondrial protease and aconitase activities were increased in Hsp22 over-expressing flies. While mitochondrial protease are involve in the clearance of oxidized proteins, aconitase is an iron-sulfure cluster TCA cycle enzyme that is highly sensitive to oxidative stress. Interestingly, the mitoproteome profile of Hsp22 over-expressing flies displayed many differences in TCA and ETC enzymes expression comparatively to controls, suggesting different modulation of these important pathways. Altogether, the results suggest a role of the small heat shock protein in the maintenance of mitochondrial homeostasis and functions. (Supported by the Canadian Institutes of Health Research and studentship from PROTEO)

PPA08

Free-radical first responders: The characterization of MnSOD and 2 CuZnSOD regulation during freezing of the freeze-tolerant North American 3 wood frog, Rana sylvatica (syn Lithobates sylvaticus)

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The North American wood frog, Rana sylvatica (syn Lithobates sylvaticus), is able to overcome subzero conditions through overwintering in a frozen state. Freezing imposes ischemic and oxidative stress on cells as a result of cessation of blood flow. Superoxide dismutases (SOD, E.C. 1.15.1.1) are antioxidant enzymes that catalyze the redox reaction involving the dismutation of superoxide (O2-) to molecular oxygen and water. The present study investigated purified CuZnSOD and MnSOD from the muscle of control and frozen R.sylvatica. CuZnSOD from frozen muscle showed a significantly higher Vmax (1.52 fold) in comparison to CuZnSOD from the muscle of control frogs. MnSOD from frozen muscle showed a significantly lower Km for O2- (0.66 fold) in comparison to CuZnSOD from the muscle of control frogs. Total phospho-staining of MnSOD, along with investigation using antibodies for phosphorylation on specific residues, showed post-translational modification via reversible phosphorylation on serine and tyrosine residues

may be responsible for the kinetic modification as the frozen form of MnSOD showed more phosphorylated serine (2.36 fold) and tyrosine (1.27 fold) residues in comparison to MnSOD purified from control animals. The stability of CuZnSOD and MnSOD was assessed using pulse proteolysis. The susceptibility to digestion via thermolysin after incubation with increasing amount of urea (Cm) was tested, resulting in no significant changes for CuZnSOD between control (0.62 M) and frozen (0.72 M) frogs, whereas a significant change in MnSOD stability was observed between control (2.51 M) and frozen (2.92 M) frogs. A partial nucleotide sequence of CuZnSOD and MnSOD from R. sylvatica were obtained using homology based PCR. The expression of CuZnSOD and MnSOD was quantified at both gene and protein levels in the muscle of the frog. RT-PCR showed no change in CuZnSOD or MnSOD transcript levels in the muscle tissue of frozen versus control animals. Protein levels, confirmed via western-blotting using cross-reactive antibodies, revealed no significant changes in protein level for either CuZnSOD or MnSOD in the frozen state. The physiological consequence of freeze-induced SOD modification appears to adjust SOD function to account for rising oxidative stressors in freezing frogs.

PPA09

Analyzing the substrate specificity of human N-acetyltransferase 8 for aromatic cysteine conjugates

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Background: The mercapturic acid pathway is a metabolic route for the detoxication of xenobiotics, processing drugs and toxicants to mercapturic acids (N-acetylcysteine conjugates). In this pathway, first, glutathione transferase catalyzes the conjugation of xenobiotic electrophiles to glutathione. Gamma-glutamyltransferase catalyzes the hydrolysis of the γ-glutamyl group, forming a cysteinylglycine conjugate. Dipeptidases catalyze the hydrolysis of cysteinylglycine conjugates, resulting in cysteine conjugates. Finally, an N-acetyltransferase enzyme, NAT8, catalyzes the transfer of an acetyl group from acetyl-CoA to the cysteine amino group, producing a mercapturic acid, which is excreted in the urine. Human NAT8 was recently cloned and expressed (Veiga-da-Cunha et al., J. Biol. Chem. 285: 18888-18898, 2010), but little is known concerning its activity and specificity with regard to substrates of toxicological importance. Objectives: The goal of this study is to characterize the specificity of NAT8 for aromatic cysteine conjugates. Methods: Few cysteine conjugates are commercially available; they will be prepared in-house. We have synthesized S-(1-menaphthyl)-L-cysteine and the corresponding mercapturic acid. The NAT8 expression plasmid was the kind gift of Dr. M. Veiga-da-Cunha. Human NAT8 was expressed in HEK 293T cells. Colorimetric enzyme assays will be performed using 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) to measure the levels of CoASH product (absorbance at 412 nm). HPLC analysis will also be used to measure the aromatic substrates and products. Results: Conjugate synthesis: The identities of the synthetic S-(1-menaphthyl)-L-cysteine and the corresponding mercapturic acid were confirmed by electrospray mass spectrometry. Enzyme expression: The presence of the NAT8 open reading frame on the expression plasmid was confirmed sequencing. Following transfection of HEK 293T cells, NAT8 expression was confirmed by western blot analysis with an anti-NAT8 antibody. Enzyme activity: Transfected HEK293T cell lysate showed activity with S-benzyl-cysteine, whereas the non-transfected control lysates were inactive. The specific activity of the lysate was 2.45 nmol product formed per min per mg protein. Conclusions: For analysis of NAT8 activity for acetylation of the aromatic cysteine conjugates, colorimetric and HPLC methods will be used, following previous work in our laboratory (Agblor and Josephy, Chem.-Biol. Interact. 203: 480-485,

2013). Enzyme kinetic experiments will be performed. Additional aromatic cysteine conjugates will also be synthesized. This information will lead to a greater understanding of the specificity of NAT8 for cysteine conjugates of toxicologically significant xenobiotics, such as polycyclic aromatic hydrocarbons. We wish to thank NSERC Canada for support.

PPA10

The role of transcription factor Nrf2 in mild thermotolerance induced at 40°C

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Hyperthermia (or heat shock) is a recent cancer treatment used in combination with chemotherapy and/or radiotherapy. These classical treatments are cytotoxic and can also cause fevers in patients at temperatures such as 39-40˚C. Fever temperatures can lead to the development of mild thermotolerance, a phenomenon that can induce resistance to heat shock-induced apoptosis. Thermotolerance is transient so its impact on thermotherapy is not an issue. Its interest lies in its ability to protect a cell against various stresses (ER stress, oxidative stress) leading to activation of the different pathways of apoptosis. We have shown that thermotolerance induced during at 40°C protects HeLa cells against apoptosis induced by hyperthermia at 42 or 43°C. There was a decrease in several markers of apoptosis (caspase activation, chromatin condensation) in thermotolerant cells compared to the non-thermotolerant cells, confirming their increased resistance to heat stress. Furthermore, mild thermotolerance protected cells against other forms of stress, for example oxidative stress. To improve understanding of the mechanisms involved in mild thermotolerance at 40°C, we investigated the role of oxidative stress and the NF-E2 related factor 2 (Nrf2) pathway. We showed via FACScan and fluorescence microscopy that the levels of several ROS increased gradually between 5 min and 3h of exposure at 40°C. The level of the Nrf2 protein increased in the nucleus of thermotolerant cells after only 5 min at 40°C, suggesting the activation of this pathway as an early event in the development of mild thermotolerance. The improved understanding of thermotolerance and the wider context of its protective effect could lead to its use as a treatment against various stress-related diseases.

PPA11

Age-dependent decline in aerobic glycolysis correlates with memory loss in a transgenic mouse model of Alzheimer's disease

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The majority of glucose consumed by the adult brain is fully oxidized to carbon dioxide and water in the mitochondria of neurons to supply the large amounts of ATP required for synaptic transmission. However, a certain percentage of glucose in the brain is exclusively metabolized by glycolysis, even in the presence of oxygen, with the generation of lactate as a by-product. This process is known as aerobic glycolysis. Emerging evidence now suggests that aerobic glycolysis in the brain plays a critical role in generating biosynthetic metabolites during early childhood development and persists in certain regions of the adult brain to support synaptic plasticity, learning and memory. However, aerobic glycolysis steadily declines with age and virtually disappears in the elderly. Our lab has recently demonstrated that metabolic reprogramming toward aerobic glycolysis confers a survival advantage to nerve cells against the toxic effects of amyloid beta, a key pathogenic peptide in Alzheimer's disease (AD). Neuronal cells with elevated aerobic glycolysis also exhibited a marked reduction in mitochondrial-derived reactive oxygen species and a reduced propensity to undergo apoptosis. In this study, I demonstrate that a progressive decline in aerobic glycolysis occurs in the mouse brain with age and correlates with a loss of spatial learning and memory. Proton magnetic resonance spectroscopy revealed an age-dependent decline in

cortical lactate levels in wild-type mice. Western blot analysis of cortical extracts revealed a decline in key regulatory proteins of aerobic glycolysis in both control and, to a greater extent, transgenic AD mice at 12 months of age when compared to tissue from younger mice. The decline in aerobic glycolysis regulators correlated with the onset of memory loss in transgenic AD mice as measured by the Morris water maze. In addition, immunoblot analysis of cortical tissue from non-demented individuals with AD neuropathology (NDAN) exhibited elevated markers for aerobic glycolysis as compared to age-matched AD patients or control individuals. These data indicate that aerobic glycolysis in the brain declines normally with age, which may contribute to neurodegeneration and memory loss associated with aging and AD.

PPA12

Using toxicogenomics to identify a primary role of oxidative stress in the mode of action of hepatocarcinogenesis for the food contaminant furan

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Typically identification and assessment of chemical carcinogens is accomplished using the 2-year rodent cancer bioassay (RCB) and a standard battery of in vitro genotoxicity assays. The former is time and resource intensive, and the latter cannot detect chemical carcinogens with non-genotoxic modes of action (MOAs). Toxicogenomics, the study of gene expression changes following chemical exposure, is currently being explored as an alternative testing strategy because it is quicker, cheaper, uses fewer animals, and can detect both non-genotoxic and genotoxic MOAs. We conducted a toxicogenomics case study using the hepatocarcinogen furan in order to explore the potential utility of gene expression data in human health risk assessment. Human exposures to furan are usually via heat-treated foods or combustion emissions. Furan has previously been tested in the RBC and thus is an ideal case study chemical since gene expression changes can be phenotypically anchored to histopathological changes and cancer outcome. Further, furan's cancer MOA – chronic cytotoxicity followed by sustained proliferative regeneration – is not detectable by the genotoxicity battery since it is non- or indirectly-genotoxic. We used DNA microarrays to derive gene expression profiles from the liver tissue of female B3C6F1 mice exposed to non-carcinogenic (0, 1, 2 mg/kg bw) or carcinogenic (4 and 8 mg/kg bw) levels of furan for 3 weeks. Furan's bioactivation to the cytotoxic metabolite cis-2-butene-1,4-dial (BDA) by cytochrome P450 2E1 (CYP2E1) is known to produce reactive oxygen species and deplete glutathione. We saw many indicators of oxidative stress in our gene expression data, most notably the enrichment of the Nrf2 Oxidative Stress Response pathway. We modeled the dose-response of this pathway and, remarkably, the benchmark dose (i.e.: the dose associated with a change in or point of departure of a biological effect) for activation of the Nrf2 pathway at 3 weeks was 2.25 mg/kg bw, which was highly predictive of furan's carcinogenic dose range (2-4 mg/kg bw) at 2 years. Since chronic activation of Nrf2 has been shown to confer a survival advantage to pre-cancer cells, we postulate that a key event in the furan cancer MOA is metabolism-related production of oxidative stress leading to chronic activation of Nrf2, which facilitates carcinogenic transformation. We also observed upregulation of genes and enrichment of pathways that were consistent with the previously observed cytotoxicity/regenerative proliferation phenotypes. Taken together, our furan case study shows that toxicogenomic analysis of sub-chronically-exposed tissues can be predictive of chemically-induced cancer phenotypes, can reveal novel oxidative stress signatures of chemical carcinogens, and can inform quantitative risk

assessment, indicating that it is a promising alternative to standard testing approaches.

PPA13

***In utero* undernutrition in mice lowers mitochondrial reactive oxygen species emission in skeletal muscle fibers of adult offspring**

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It is well established that there is an association between in utero undernutrition and risk for obesity, insulin resistance, type 2 diabetes and cardiovascular disease. However little is known about specific effects in skeletal muscle. Previous results from our group showed that in utero undernutrition in mice alters characteristics of energy metabolism in isolated mitochondria and permeabilized fibers from skeletal muscles (Beauchamp et al, Under review). Effects on muscle mitochondrial energetics were associated with decreased whole body energy expenditure and decreased mitochondrial content in muscles having mixed fiber types. Using Amplex Red fluorimetry in O2K incubation chambers (Oroboros; Innsbruck, Austria) we analyzed reactive oxygen species (ROS) emission in red and in white gastrocnemius muscles of female mice at 14 weeks of age. Results in white gastrocnemius muscle fibers showed a significant decrease in ROS emission (normalized to citrate synthase activity, p less than 0.05); a total mean of 44.1% decrease in ROS emission was observed between the undernourished muscles and the untreated muscles during states 2 and 3 of oxidative phosphorylation. ROS emission during malate-driven respiration was 43.1% lower in fibers from the offspring of undernourished mice compared to control mice (from ad libidum fed dams). ROS emission during respiration fuelled by octanoyl carnitine, ADP, pyruvate and glutamate was 46%, 44.6%, 42.3% and 44.7% lower (all; p less than 0.05), respectively, compared to controls. Intriguingly no effects on ROS emission profiles were observed in fibers prepared from red gastrocnemius. Western blots of superoxide dismutase and Oxyblot determinations of total protein carbonyls are being conducted in red and in white gastrocnemius muscles in order to better understand these surprising decreases in ROS emission. Once deciphered, these observations will shed more light on the effects of in utero undernutrition on ROS and oxidative stress in skeletal muscle.

PPA14

Cytoprotective mechanisms of isoniazid in promyelocytic leukemia (HL-60) cells

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Isoniazid (INH) is one of the oldest and successful anti-tuberculosis drugs used; however, it appears to exert multiple effects through different pathways. Although INH is known to cause adverse drug reactions (mainly hepatotoxicity) in some individuals, there could be beneficial effects of INH. Therefore, the objective of this study was to investigate the cytoprotective mechanisms of INH on HL-60 cells that are challenged with H_2O_2. Several studies have suggested that H_2O_2 induced HL-60 cell death is caspase-independent (termed pyknosis) where apoptotic-inducing factor (AIF) moves to the nucleus from mitochondria to induce chromatin condensation and cell death. We hypothesized that INH induces cytoprotection by ATP synthesis as well as through its inhibition of myeloperoxidase (MPO); these actions would stimulate the growth phase of the cell cycle and attenuate oxidative stress, respectively. In our cell viability studies, we found that INH was cytoprotective against H_2O_2 induced cytotoxicity in HL-60 cells in a concentration-depedent manner. Using quantitative proteomics, we found highly significant changes in 51 proteins, among which 18 were upregulated and 33 were

downregulated. These proteins revealed cellular pathways that could signal for enhanced ATP generation and cell-cycle regulation. On the other hand, we found a large number of INH-protein adducts through immunoblots, and these adducts were enhanced in the presence of H_2O_2, even though INH conferred cytoprotection. As INH is a partial MPO inhibitor, these adducts may have been formed through pathways other than the peroxidase activity of MPO. Our future studies will be aimed at identifying INH-protein adducts and their role in cytoprotection. This study will be able to find out important cellular pathways of cytoprotection of INH against oxidative stress which is well-known for myeloid leukemia diseases.

PPA15

Glutathione-mediated effects of lithium in decreasing protein oxidation induced by mitochondrial complex I dysfunction

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Mitochondrial complex I dysfunction could result in increased production of reactive oxygen species, resulting in protein oxidation and nitration. Complex I dysfunction and increased markers of oxidative stress are two of the most consistent findings in bipolar disorder (BD), which is a psychiatric disorder characterized by alternating episodes of mania and depression. Lithium, which is the most commonly prescribed drug for the treatment of BD, is known to have antioxidant properties, which may occur through the glutathione system, an intrinsic antioxidant. Therefore, we aimed to elucidate whether glutathione is involved in lithium's ability to decrease protein carbonylation and nitration produced by complex I inhibition. Neuroblastoma cells (SH-SY5Y) were treated with rotenone (5-30nM) to model complex I dysfunction in BD. Complex I inhibition resulted in an increase in protein nitration ($F_{5,36} = 9.58$, P less than 0.01) and carbonylation ($F_{5, 24} = 15.38$, P less than 0.01), and decreased cell viability ($F_{5,24} = 4.09$, P less than 0.01). Lithium (0.75mM) pre-treatment prevented rotenone-induced complex I dysfunction (no group difference), cell mortality (no group difference), nitration (no group difference) and partially ameliorated increased carbonylation (difference between lithium pre-treated groups and medium pre-treated groups: $F_{1,48} = 22.23$, P less than 0.01). Importantly, while lithium's ability to prevent carbonylation was dependent on glutathione ($F_{3,16} = 25.10$, P less than 0.01), its effect on nitration was not. The findings of this study suggest that lithium's antioxidant effects may occur through different pathways for protein nitration and oxidation, where lithium may ameliorate carbonylation by increasing glutathione levels, but may decrease protein nitration through a different mechanism, such as by improving complex I function. Understanding the different pathways involved in oxidative stress in BD may reveal potential targets for the development of novel interventions for this disease.

PPA16

Infant birth weight and maternal physiology: endothelinergic system and oxidative stress

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Maternal physiological changes due to factors including environmental chemical exposures can precipitate adverse birth outcomes such as low infant birth weight. Low infant birth weight has been associated with increased rates of early onset of adulthood diseases. Understanding maternal biological pathways, their potential modulation by environmental exposures and their impact on progression of pregnancy will contribute to reducing the risk of adverse pregnancy outcomes. In this study, our objective was to investigate associations among third trimester maternal plasma biomarkers of endothelial dysfunction and oxidative stress, maternal physiological correlates, and birth weight to identify adverse outcome pathways. Third trimester plasma samples from N=144 mothers were analysed for vasoregulatory endothelin isoforms (e.g. BET-1) by a HPLC-Fluorescence method. Other target markers of vascular health namely cellular adhesion molecules (e.g. VCAM), chemokines (e.g MCP-1), vascular endothelial growth factor (VEGF) and matrix metalloproteinases (e.g MMP-2) in these samples were analysed by an affinity-based multiplex protein array method. A lipid oxidation marker 8-isoPGF2alpha was measured in plasma by a competitive enzyme immunoassay. Statistical tests for associations among the different maternal parameters and birth weight and maternal blood pressure included Pearson correlations and backward stepwise regression analyses. Biomarkers of vascular integrity and inflammation exhibited changes characteristic of the infant birth weight range. In general, infant birth weight was significantly associated with VEGF (r=0.33) and MMP-9 (r=-0.27). Similarly, maternal BP (systolic) was associated with MMP-9 (r=0.19), whereas BP (diastolic) was correlated with CRP (r=0.23) and MMP-1(r=0.17). Gestational age and BET-1 were predictive of low infant birth weight. Furthermore, the lipid oxidation product 8-isoPGF2alpha was positively associated (r=0.26) with ET-1, a potent vasoregulatory peptide. In conclusion, vascular mechanisms and oxidative stress can impact on maternal physiology and thus on pregnancy outcome.

PPA17

Besting Vitamin E: Sidechain Substitution is Key to the Reactivity of Novel Naphthyridinol Antioxidants

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A series of naphthyridinol analogs of α-tocopherol (α-TOH) with varying sidechain substitution was synthesized to determine how systematic changes in the lipophilicity of these potent antioxidants impact their radical trapping activities in lipid bilayers, regenerability by water-soluble reductants, binding to human tocopherol transport protein (TTP), cytotoxicity and radical trapping activities in mammalian cells. The activities of the naphthyridinols were assayed in phosphatidylcholine unilamellar liposomes using a recently developed high-throughput fluorescence assay. The naphthyridinols afforded a dose-dependent protection of the fluorescent probe consistent with unprecedented peroxyl radical-trapping activity in lipid bilayers. While sidechain length and/or branching had no effect on their apparent reactivity, it dramatically impacted reaction stoichiometry, with more lipophilic compounds trapping two peroxyl radicals and more hydrophilic compounds trapping significantly less than one. These results were corroborated by measurements in multilammelar liposomes wherein formation of lipid hydroperoxides were monitored directly using a recently developed

coumarin–triarylphosphine conjugate that undergoes fluorescence enhancement upon reaction with hydroperoxides. The cooperativity of a lipophilic naphthyridinol with water-soluble reducing agents was also studied in liposomes and revealed superior regenerability by each of ascorbate, N-acetylcysteine, and urate when compared to α-TOH. Binding assays with human TTP, a key determinant of the bioavailability of the tocopherols, revealed that the naphthyiridinols with sidechains of eight or more carbons had affinities for TTP which were similar to, and in one case 10-fold better than, α-TOH. The activities of naphthyridinols as inhibitors of lipid peroxidation were assayed in human erythroblasts, revealing that more lipophilic naphthyridinols have similar efficacies with α-TOH at less than 1 μM, however, more hydrophilic naphthyridinols did not show any activity. The cytotoxicity studies revealed that more lipophilic naphthyridinols induce cell death at concentrations lower than more hydrophobic naphthyridinols. It is suggested that the less lipophilic compounds autoxidize rapidly in the aqueous phase and that preferential partitioning of the more lipophilic compounds to the liposome and mammalian cells protects them from autoxidation. This hypothesis was further supported by that fact that the acetate protected lipophilic naphthyridinol provided better stability and inhibition activities of lipid peroxidation in mammalian cells.

PPA18

Identification of a Role for Uncoupling Protein 3 in Modulating Mitochondrial Superoxide Flashes

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Mitochondria are a major site of reactive oxygen species (ROS) production in cells. While ROS can cause oxidative damage in cells, they are also vital in many signaling processes. One means through which ROS participate in signaling is through the oxidation of protein thiols. ROS exist in many molecular forms, however the proximal form emitted from the electron transport chain is superoxide. Recently, the identification of mitochondrial superoxide flash (mSOF) events was sparked through sensitive measurements of temporal and spatial differences in superoxide production using a mitochondrial targeted protein biosensor, mt-cpYFP. mSOF are stochastic events of quantal bursts in superoxide production, which occur concomitantly with transient mitochondrial matrix depolarization events. Furthermore, the characteristics of these metabolically linked events have been defined in a variety of cells types. While initial theories surrounding the mechanistic control of these events involved the opening of the mitochondrial permeability transition pore, conflicting evidence exists. Intriguingly, all cell types in which mSOF have been characterized express a member of the mitochondrial uncoupling protein (UCP) family. While still controversial, the UCPs are thought to be activated by ROS, and thereby uncouple respiration from ATP synthase activity. By lowering mitochondrial membrane potential, UCPs are thought to decrease ROS emission. Recently, we have identified a possible role for UCP3 in modulating characteristics of mSOF. While some mSOF characteristics were unchanged in the absence of UCP3 expression, analyses showed a prolonged duration of flashes in the UCP3KO muscle, 22.04s, compared to WT muscle, 21.17s ($p=0.04$). Additionally, the average area of the flashes in UCP3KO mice was 30% smaller than that observed in WT mice (p less than 0.01). We have unearthed a novel relationship between flash amplitude and mitochondrial depolarization and with the use of spinning disk confocal technology we are able to increase the temporal resolution while imaging to discern the order of these events during mSOF further elucidating the mechanism of this phenomenon. Investigations of the similarities and differences in mSOF characteristics among various muscles are currently on going. Knowledge of the key regulators of mSOF events will allow the future possible elucidation of mSOF signaling roles in cellular processes.

PPA19

Effect of a complex dietary supplement on radiation induced oxidative stress and apoptosis in the heart

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High doses of whole body radiation has been shown to lead to cardiac stress and cardiovascular disease. We have recently showen a complex dietary supplement consisting of multiple anti-oxidants and anti-inflammatories can increase longevity and decrease markers of ageing in mice and impart protection against radiation induced oxidative stress and inflammation in mouse bone marrow and lymphocytes, but it's effects in the heart have not been investigated. The specific aim of this research is to test the hypothesis that a complex dietary supplement can protect the mice heart from a high dose of ionizing radiation. C57BL/6J male mice were fed a complex dietary supplement for 30 days prior or after a single whole body 5Gy dose of radiation. We are currently examining the protein levels of antioxidants MN-SOD and Catalase, and apoptosis markers Caspase 3, Bax and Bcl-2 in the mice hearts. Recently, oxidative stress has been shown to play a role in autophagy, though the significance of autophagy in the heart is not clear; autophagy has been reported to protect against cell death as well be the cause of cell death. The role of radiation induced autophagy in the heart has not been investigated. We are also examining the protein levels of autophagy marker LC3b in the heart after whole body radiation and the effect of a complex dietary supplement. Findings from these studies will reveal if a complex dietary supplement can provide protection to the heart from radiation induced oxidative stress and cell death.

PPA20

Towards Isoform-Specific Lipoxygenase Inhibitors: Fluorinated Polyunsaturated Substrate Analogs and Acetaminophen-Inspired Compounds

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Oxidative metabolism of arachidonic acid (AA) by lipoxygenase (LOX) leads to the formation of leukotrienes. Under patho-physiological conditions leukotrienes have been linked to platelet aggregation, inflammation, and adverse smooth muscle contraction. Inhibition of leukotriene biosynthesis has been extensively studied as a potential for the development of novel therapies for inflammation, and respiratory diseases in particular, asthma. Very few inhibitors for LOX have been developed, the main challenge being 6 different isoforms of the enzyme and it is unclear how different isoforms oxygenate the PUFAs with high stereo and regio-specificity. Current efforts focus on synthesizing non-natural substrate mimics that are resistant to oxidation by LOX. One approach is the synthesis of fluorinated linoleic acid, where the fluorine atoms serve as bioisostere of hydrogen and prevent metabolism by the enzyme. This would allow for the crystallization of LOX in its active conformation without substrate degradation. Crystallography information would be an asset in determining the structure of protein complex in the active site and gain further insights on the mechanism of action. This would help design isoform specific inhibitors for different LOX in the body. Small molecule inhibitors of LOX inspired by ApAP inhibitory effect on COX are also being studied. While the inhibitory activity of COX is due to its redox chemistry, preliminary studies on different pyrimidine analogues of ApAP suggest that LOX inhibition occurs via a different pathway. Preliminary results obtained suggest that the bi-dentate coordination of ferric iron in the active site is responsible for the inhibitory activity. Modeling studies of LOX are underway to design better inhibitors and elucidate the mechanism of inhibition. With modeling results in hand, the structure-activity of the pyrimidine ApAP analogues can be modified to obtain isoform specific inhibitors of LOX.

PPA21

UVC-induced a subclass of cytoplasmic RNA granules in mammalian cells

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Stress granules (SGs) are well characterized cytoplasmic RNA bodies that form under various stress conditions. In mammals, most SGs-inducing stresses prevent general translation suggesting that formation of these RNA containing bodies is a consequence of reduced rate of translation initiation. We tested this hypothesis using ultra-violet C (UVC) irradiation at non-lethal doses. We have observed that exposure of mammalian cells in culture to UVC induces the formation of discrete cytoplasmic RNA granules that we termed mammalian UVC Granules (mUVCGs). The presence of these granules seems to occur independently of eIF2α phosphorylation and, importantly, does not correlate with major translation inhibition. Concomitant with the accumulation of mUVCGs in the cytoplasm, cells enter a quiescent state, as they are arrested in G1 phase of the cell cycle. This block persists as long as mUVCGs are present. A tight correlation between mUVCGs decay and re-entering into S-phase was observed. UVC-induced cytoplasmic granules are not Processing Bodies (P-bodies) and seem a priori to be stress granules (SGs). However the kinetics of their formation, their absence of fusion into large granules, their persistance over 48 hours and their slow decay, all point to the possibility that they belong to a subclass of granules which function's might differ from the classical SGs.

PPA22

An 'HO-1 Transducer' Model of Schizophrenia

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Neural development is highly susceptible to disruption by oxidative and other stressors. The discovery of key molecules that act epigenetically to transduce various stress stimuli into aberrant patterns of central nervous system development may suggest new strategies for the treatment of neurodevelopmental disorders such as schizophrenia. Selective overexpression of the stress protein heme oxygenase-1 (HO-1) in astrocytes of novel GFAP.HMOX1 transgenic mice results in subcortical oxidative stress and mitochondrial damage/autophagy; increased iron deposition in numerous brain regions; diminished neuronal reelin and GAD67 content (males); induction of Nurr1 and Pitx3; increased tyrosine hydroxylase and α-synuclein expression; augmented dopamine and serotonin levels in basal ganglia; reduced D1 receptor binding in nucleus accumbens; axodendritic pathology; altered hippocampal cytoarchitectonics; enlarged lateral ventricles; impaired neurovascular coupling; attenuated prepulse inhibition (males); and hyperkinetic behavior. The GFAP.HMOX1 neurophenotype bears resemblances to schizophrenia and other neurodevelopmental conditions and implicates astroglial HO-1 as a prime transducer of diverse stressors into altered neurodevelopmental trajectories. Containment of the glial HO-1 response to stressors at strategic points of the life cycle may provide a novel approach for the prevention or management of schizophrenia and other human neurodevelopmental conditions.

PPA23

Over-expression of Heme Oxygenase-1 (HMOX1) Regulates microRNA Expression in Rat Astrocytes

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Background: MicroRNAs (miRNAs) are noncoding RNA species of 20 to 25 nucleotides in length and bind to the 3' untranslated region (3'-UTR) of target genes to cause targeted mRNA cleavage or degradation or protein translation repression. Dysregulated miRNA expression may contribute the etiopathogenesis of neurodevelopmental and neurodegenerative disorders. We previously demonstrated that over-expression of the heme-degrading enzyme, heme oxygenase-1 (HO-1) promotes iron deposition, oxidative mitochondrial damage and autophagy in astrocytes and enhances the vulnerability of nearby neuronal constituents to oxidative injury. Objective: To determine whether altered patterns of miRNA expression participate in HMOX1-related glial injury. Methods: MiRNA microchip assays were performed on HMOX1-transfected primary astroglial cultures and altered miRNAs were further validated with qPCR. The effects of heme degradation products on miRNA expression were assessed and salient mRNA targets of the impacted miRNAs were ascertained. Results: In HMOX1-transfected astrocytes, rno-miR-140*, rno-miR-17, and rno-miR-16 were significantly elevated, and rno-miR-297, rno-miR-206, rno-miR-187, rno-miR-181a, rno-miR-138 and rno-miR-29C were down-regulated compared to sham-transfected controls. The heme degradation products, carbon monoxide and ferrous iron largely recapitulated the HMOX1 effects, whereas bilirubin appeared inert in this regard. The mRNA expression of Ireb, Ngfr, Mapk3, Tnf, and Sirt1, known targets of the down-regulated miRNAs, was significantly increased in HMOX-1-transfected astrocytes relative to sham-transfected preparations (p less than 0.05-0.01). Conclusions: A model implicating altered expression of miRNAs and certain targeted mRNAs in HMOX1-related glial damage is proposed.

Poster Presentation Abstracts
Poster Session II

Thursday, June 12, 2014
11:45 AM – 1:30 PM
River Building Atrium

PPA24
Investigating the Redox Potential of Parkin
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BACKGROUND: Recessive Parkinson's Disease (PD) is a dopaminergic cell-specific neurodegenerative disease which affects >110,000 Canadians and is the second most prevalent neurodegenerative disease. Early-onset PD is caused by autosomal recessive mutations in the DJ-1, PINK1 and PARK2 genes. Unfortunately, no specific role for Parkin has been validated, however, oxidative stress (OS) alters both, E3 ligase activity and mitophagy. Unbiased proteomic studies in park2 -/- mice corroborate the concept of increased OS. The present research focuses on understanding Parkin's role in protecting cells from OS. HYPOTHESIS: Having a large number of cysteines, which are susceptible to oxidation, we propose that Parkin is able to protect cells from OS by regulating redox changes and accomplishes this by altering the redox state of its own thiol groups. METHODS: Parkin's ability to indirectly and directly reduce H_2O_2 is determined using two independent methods: chemiluminescence and HyPer assay. The former consists of measuring the luminescence produced from the oxidation of luminol in the presence of H_2O_2 and horseradish peroxidase. Maltose-Binding Protein (MBP)-tagged recombinant human parkin is introduced into this reaction medium and changes are quantified. The HyPer in vitro assay measures the shift in the excitation/emission of an YFP-labelled redox-sensitive protein (OxyR) expressed in HEK293 cells. Transient human FLAG-tagged Parkin expression is induced and compared to vector control. RESULTS: Both the chemiluminescence and the HyPer assay have been optimized and proven functional with positive and negative controls. Preliminary data (n=2) suggest that FLAG-Parkin is able to buffer H_2O_2-induced redox changes in cell lysates.

Selected Publications: Kitada T, et al. Nature. Apr 9 1998., Shimura H, Schlossmacher MG, et al. Science, Jul 13 2001., Palacino JJ, Sagi D, Goldberg MS, et al. The Journal of Biological Chemistry. Apr 30 2004., Jones DP. American journal of physiology. Cell physiology. Oct 2008., Matés, et al. Clinical Biochemistry, 1999., Muller CH, et al. Spermatogenesis: Methods and Protocols, Methods in Molecular Biology. 2013;927:363-378., Belousov, VV, et al. Nature Methods, April 2006., LaVoie, Schlossmacher, et. al. Journal of Neurochemistry, 2007.

PPA25

The Reactions of Radical-Trapping Antioxidants with Peroxyl Radicals in Ionizing Media

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Under thermal and photolytic conditions, (E)-tert-butyl 4-(naphthalen-2-yl)but-3-eneperoxoate undergoes homolytic O-O bond cleavage to form an alkoxyl and acyloxyl radical. The latter rapidly decarboxylates to form a carbon-centered radical that has been shown by our group to serve as a precursor to a powerful peroxyl radical clock in the presence of oxygen (1,2). As a competitive kinetic approach, this method allows the rate constants for H-atom transfer reactions between peroxyl radicals and antioxidants to be easily determined in an array of solvents, simply using GC or HPLC. To date, the peroxyl radical clock has been used to study the kinetics of peroxyl trapping by antioxidants in non-polar solvents. While non-polar solvents are appropriate models for the inside of lipid bilayers and lipoproteins where lipid peroxidation takes place, it may be inappropriate to assume that the kinetics and mechanisms of reactions of peroxyl radicals are the same in the cytosol or aqueous compartments of the cell, where peroxyl radicals are often invoked as intermediates in a variety of processes, including radical-mediated damage to DNA, proteins and carbohydrates. We demonstrate that the peroxyl radical clock approach is useful for the study of peroxyl radical reactions in polar (ionizing) media. Herein we will describe herein the calibration of a peroxyl radical clock in methanol and its application to study the kinetics and mechanism of reaction of peroxyl radicals with phenolic radical-trapping antioxidants. We show for the first time that acidic phenolic antioxidants react with alkylperoxyl radicals by a sequential proton loss electron-transfer mechanism (SPLET) and not by a hydrogen atom transfer (HAT) mechanism, as has been assumed.

1. Jha, M.; Pratt, D. A. Kinetic solvent effects on peroxyl radical reactions. Chem. Commun. 2008, 1252-1254; 2. Hanthorn, J. J.; Pratt, D. A. Peroxyesters as precursors to peroxyl radical clocks. J. Org. Chem. 2012, 77, 276-284.

PPA26

Antioxidant activity of oat bran digested proteins and fractions from high performance liquid chromatography

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Introduction: Macromolecules come in four classes, carbohydrates, proteins, lipids and nucleic acids. Recent research has focused on the potential of these macromolecules to provide the human body with biological activity apart from their basic nutritional values. Intact proteins possess biological activity; however, their large size might hinder their absorption and permeability through cell membranes. Meanwhile digestion of food proteins by fermentation or proteolysis has the potential liberate short chain oligopeptides with in vivo activity (Walther and Sieber 2011) (Meisel H. 2004). In this study, antioxidant activity of Protamex hydrolyzed oat bran proteins and HPLC-separated fractions investigated for the first time. Methods: Oat bran proteins were digested with Protamex. Reverse phase high performance liquid chromatography (RP-HPLC) was used to separate protein hydrolysates on a C18 column to eight peptide fractions. Peptide fractions and digested proteins were tested for their antioxidant activity against peroxyl, superoxide anion and hydroxyl radicals. Moreover, chelating activity was also determined. Results: Eight fractions (F1-F8) were obtained from separation of the oat bran protein hydrolysate (OPH). Antioxidant activity was measured using four different assays. In oxygen radical absorbance assay (ORAC), F7 had highest peroxyl radical quenching activity (844.4 uM

TE/g) whereas F1 and F2 had the lowest (27.9 and 39.1 uM TE/g, respectively). An increase in ORAC values F1 to F7 suggests the possibility for relation between the degree of hydrophobicity and activity. In the superoxide anion radical assay F3 and F6-F8 had higher activities (48-51% inhibition) while in the hydroxyl radical assay, the activity of F4, F7 and F8 were the highest (14-16% inhibition). Both assays showed no relation with hydrophobicity. In metal chelation F7 had the best iron chelating activity (22% inhibition). Raman spectroscopy and LC-MS/MS are been performed to determine spectroscopic characteristic and sequences that may influence the activity. Conclusion: Results from this study have shown that fractions F6-F8 to be most active; F7 had the highest activity among the assays. The identification of the peptide will provide data for investigation of structure-function properties. Acknowledgment: This work was supported by Ministry of higher education Saudi Arabia King Abdullah Scholarship program to Morooj Baakdah.

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PPA27

Antioxidant defense in an anoxia-tolerant mollusc: The role of hexokinase and glucose-6-phosphate dehydrogenase regulation in increasing the potential for NADPH production.

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The marine mollusc, *Littorina littorea*, is an intertidal snail that experiences daily bouts of anoxia/hypoxia with the tide cycle. During these cycles these marine snails will experience dramatic fluctuations in oxygen availability with increases in free radical production occurring when snails transition from open air to water submersion. The increase in free radicals necessitates good antioxidant defense mechanisms, which includes the continued production of NADPH within the cell. Hexokinase and glucose-6-phosphate dehydrogenase (G6PDH) are two important players in mediating NADPH production as they gate the entry of glucose-6-phosphate into the pentose phosphate pathway, which is responsible for NADPH production. In the marine snail hepatopancreas, a reduction in the Km glucose and Km G6P, for hexokinase and G6PDH respectively, was observed for the enzyme originating from the anoxic animal in comparison to the enzyme from control animals. Furthermore, G6PDH from the anoxic animal displayed a significantly reduced Vmax when compared to the enzyme from the control animal. The mechanism behind the changes in kinetic parameters for both hexokinase and G6PDH was determined to be reversible phosphorylation. Both enzymes are present in a low-phosphate form during anoxia and likely act together to shunt G6P into the pentose phosphate cycle for the continued production of NADPH.

PPA28

Towards a Molecular Level Understanding of the Biological Activity of Allicin, Petivericin and other Organosulfur Compounds

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Garlic and petiveria, two plants from the Allium genus, are known to possess great medicinal value. Their potent antioxidant activity has been attributed to the thiosulfinate secondary metabolites allicin and petivericin (or S-benzyl phenylmethanethiosulfinate, BPT), respectively. In organic solvents the radical-trapping activity of thiosulfinates is paralleled by their propensity to undergo Cope elimination to yield a sulfenic acid,1 which undergoes diffusion-controlled reactions with peroxyl radicals.2,3 However, evidence recently obtained by the Pratt group suggests that in lipid bilayers,4 this mechanism becomes irrelevant due to the diffusion of the sulfenic acid product into the aqueous phase, where they cannot trap lipid-derived chain-carrying radicals. Cytotoxicity studies reveal that allicin and petivericin induce cell death at concentrations just above where they inhibit lipid peroxidation. This is not the case for a lipophilic analogue of petivericin, which shows radical-trapping activity in lipid bilayers and also in cells, at concentrations where it is not cytotoxic. These results suggest that another mechanism of action must exist for allicin and petivericin. The in vivo antioxidant ability of these thiosulfinates may be the result of reactivity towards cysteine thiol groups of signaling proteins or by glutathione depletion; both leading to the expression of antioxidant enzymes. To test this hypothesis, propargylated analogs of allicin and petivericin were synthesized, with the intention of using them as reagents for pull-down proteomic assays to identify the cellular targets of allicin and petivericin. Their reactivities to protein thiols were determined using papain and glyceraldehyde-3-phopsphate dehydrogenase as model proteins, and their reactions products determined by mass spectrometry. Progress on the identification of allicin and petivericin's protein targets and its implications will be discussed.

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PPA29

Cryopreservation of rat hepatocytes with wheat proteins: role in oxidative stress protection

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Hepatocytes are an essential model for the pharmaceutical industry, as they are used for drug toxicity testing. Their availability is dependent on the surgical isolation of fresh cells from animal liver, and cryoconservation allows for the storage of isolated cells for future use. However, the freeze/thaw step associated with this procedure leads to structural damage to the cells, which lowers post-thaw viability and metabolic functions. To circumvent such damage, cryoprotective agents such as dimethylsulfoxide (DMSO) are used, but these are often toxic for cells. The development of alternative cryoprotectants that are less toxic would therefore be beneficial. We have previously shown that wheat protein extracts are efficient for cryopreservation of hepatocytes and other cell types. To identify cryoactive proteins in these extracts, fractions from chromatographic separation were analysed by tandem mass spectrometry. Data analysis allowed us to determine that enolase is a good candidate as cryoprotector, revealing a novel function for this

glycolysis-associated protein. The cDNA was cloned and the protein was produced in a bacterial system. Viability tests confirmed that the recombinant enolase is more efficient than DMSO for cryopreservation of rat hepatocytes and that it causes no cellular toxicity. As a first step to elucidate its mechanism of action in the protection of cells, we have determined that the protein decreases the oxidative stress resulting from cryopreservation. Levels of peroxides and nitric oxide after thawing of cells frozen with enolase are lower than those in cells frozen with DMSO. These results show that this plant protein has tremendous potential as a cryoprotective agent for hepatocytes, and possibly other cell types. The use of non-toxic agents that preserve viability and metabolic functions of mammalian cells would represent a major breakthrough in the field. It would allow increasing the availability of functional cells used for toxicity testing required for medical and pharmaceutical advances.

PPA30

Protective Effect of Oat Peptides against Oxidative Stress in a Hepatic Cell Culture Model

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Introduction and objective: Oxidative stress caused by the imbalance between pro-oxidants and antioxidants in cells, is a contributor to the development of several diseases including Alzheimer's disease, cardiovascular disease, and cancer (Suja et al., 2004). A recent study found that oat proteins digested by alcalase demonstrates antioxidant activity. However, the biological activity of seven peptides FNDRLRQGQLL (P1), GLVYIL (P2), GQTV (P3), GQTVFNDRLRQGQLL (P4), YHNAP (P5), YHNAPGLVYIL (P6), and DVPNNANQLEPR (P7) identified in the digest have not been investigated. The present study aims to determine radical scavenging as well as cytoprotective activities of these peptides. Methodology: Antioxidant activities of peptides P1-P7 were determined using Oxygen Radical Absorbance Activity Capacity (ORAC) assay while the cytoprotection were on hepatic HepG2 cells using the MTT (3- [4,5-dimethylthiazol-2-yl]-2,5 diphenyltetrazolium bromide) procedure. Results: HepG2 cells were used to analyze the protective effect of peptides P1-P7 (0-200 μM) against oxidative damage induced by AAPH. P2 demonstrated the highest cytoprotection (41.7%) followed by P6 and P7. No cytotoxic effect was observed for P1-P4, P6 and P7. Surprisingly, although P5 are part of P6, treatment with P5 at 200 μM led 87% reduction in cell viability, implying the importance of both sequence and peptide length. P2, P3, P5, and P6 had higher scavenging activity than glutathione on a molar basis, as demonstrated by the ORAC assay, with P2 (0.72 Trolox Equivalent (TE)) and P5 (0.67 TE) being the most active. Although mechanisms of the two assays were different, Peptide P2, the most hydrophobic of all seven peptides, was the most active on the cell culture model and in the peroxyl radical scavenging assay (i.e. ORAC). P5 however, behaved differently in the two assays. Conclusion: The present study suggests that P1, P2, and P7 possess radical scavenging properties and cytoprotective activities against oxidative damage on HepG2 cells. Their mechanism of action is currently being investigated. References: Suja, K.P., Jayalekshmy, A. & Arumughan, C. (2004). Free radical scagenging behaviour of antioxidant compounds of sesame (Sesamumindicum L.) in DPPH system. J. Agric. Food Chem., 52, 912-915.

PPA31

Efficacy of Polyphenol-rich Potato Extracts on the Ozone-induced Pulmonary Inflammatory Response

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Ozone exposure linked with air pollution represents a significant contributor to cardiopulmonary morbidity and mortality. Biological effects of ozone are closely related to pro-inflammatory and pro-oxidative events, leading to disruption of epithelial functions in the lungs. Dietary polyphenols are associated with lung anti-inflammatory effects; however, their protection against air pollutants has not been tested. In this study, we investigated the efficacy of a polyphenol rich extract (PE) to affect two major endpoints in ozone-exposed male and female C57BL/6 mice fed a high fat Western-style diet: (1) obesity and glucose intolerance; and (2) lung inflammatory indices. Mice were fed ad libitum either a 100% PE dosage [chlorogenic acid (200 mg/kg diet) and ferulic acid (6 mg/kg diet)] or 20% PE dosage [chlorogenic acid (40 mg/kg diet) and ferulic acid (1.2 mg/kg diet)]. After 4 wk of dietary adaptation, animals were exposed to 0.8 ppm ozone or air in a stainless steel chamber for 4 h and euthanized 24 h post exposure. Independent of ozone exposure, the 100% PE dosage was effective in reducing body weight gain (79% decrease, P less than 0.001), fat pads (50% decrease, P less than 0.01) and fasting glucose levels (80% decrease, P less than 0.001) in both males and females. In comparison to ozone-exposed controls, the total inflammatory cell counts (macrophages and neutrophils) in bronchoalveolar lavage fluid relative to lung size were significantly decreased (P less than 0.005) in ozone-exposed males fed 100% PE (2.43 ± 1.04 vs. 5.57 ± 1.21), but significantly increased (P less than 0.05) in ozone-exposed females receiving 100% PE (9.40 ± 1.39 vs. 4.38 ± 0.66). Neither PE dose affected the lung inflammatory markers measured in broncho-alveolar lavage fluid (KC, Eotaxin, MIP, RANTES, IFN-γ and TNF-α). In conclusion, dietary supplementation with PE reduces: (a) adiposity and glucose intolerance associated with high fat feeding in both males and females; and (b) protects against lung recruitment of inflammatory cells associated with ozone exposure only in males, which indicates sexual dimorphism in the protection against ozone-induced lung inflammation.

PPA32

Catalytic Radical-Trapping Antioxidant Activity of Nitroxides under Weakly Acidic Conditions

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Nitroxides, such as the persistent free radical 2,2,6,6-tetramethylpiperidin-N-oxyl (TEMPO), occupy a unique position in terms of redox chemistry. This is because the radical can easily be either oxidized or reduced through one electron transfer to form a stable oxoammonium ion or hydroxylamine, respectively. As a result of this chemistry, nitroxides can be potent redox modulators, as evidenced by the potent superoxide-dismutase-mimetic activity of TEMPO and its derivatives (1). Though nitroxides react with alkyl radicals at near diffusion-controlled rates, they are poor peroxyl radical trapping antioxidants (2), and can usually only inhibit hydrocarbon (lipid) autoxidation by outcompeting molecular oxygen for chain-carrying alkyl radicals. However, we and our collaborators have shown that under acidic conditions, TEMPO can trap peroxyl radicals through proton coupled electron transfer (PCET) from its protonated radical cation (3).Remarkably, under weakly acidic conditions (e.g. in the presence of carboxylic acids), autoxidations are inhibited indefinitely by

TEMPO, in contrast with strongly acidic conditions, that result in a reaction stoichiometry of 1 (i.e. only a single peroxyl radical is trapped). The reactivity of TEMPO in the presence of weak acids imply that it is recycled from its oxoammonium ion in situ, but the nature of the reducing agent has been elusive. Here, we will provide evidence which indicates that autoxidation chain-carrying alkyl radicals are the reducing agent, thereby accounting for the exciting catalytic radical-trapping antioxidant activity of nitroxides.

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PPA33

Debunking the Involvement of Endogenous Ozone in Secosterol Synthesis within Human Atherosclerotic Arteries

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Cholesterol is one of the most abundant lipids in the body, and like all unsaturated lipids, it can be oxidized in the presence of reactive oxygen species (ROS). Lipid peroxidation is one of the main ROS-induced oxidative damages, which is linked to neurodegenerative and cardiovascular diseases. In 2003, Wentworth et. al. detected both 3β-Hydroxy-5-oxo-5,6-secocholestan-6-al (secosterol-A) and its intramolecular aldolization product 3β-hydroxy-5β-hydroxy-B-norcholestane-6β-carboxaldehyde (secosterol-B) in human atherosclerotic plaques which, at the time, were only known to be formed by cholesterol ozonolysis. However, our group has shown that cholesterol 5α-hydroperoxide, which is the product of the singlet oxygen ene reaction with cholesterol, can undergo Hock fragmentation to generate secosterol-A and -B as well. Nevertheless, the proposal of cholesterol 5α-hydroperoxide as the only precursor for secosterol-A and -B is limited because it is not particularly stable; it readily rearranges to a more thermodynamically stable cholesterol 7α-hydroperoxide. In addition, Wentworth identified secosterol-A and -B by limited characterization techniques (HPLC and MS) which cannot out rule very similar secosterol structures of similar mass. Cholesterol 7α- and 7β-hydroperoxide are known to be formed *in vivo* by cholesterol autoxidation, and formation of secosterols from these more abundant precursors would be more plausible. Upon Hock fragmentation of cholesterol 7α-hydroperoxide, two products were observed: a dialdehyde product similar to secosterol-A, and corresponding aldolization product, similar to secosterol-B. Although the products obtained are not identical to the ones reported by Wentworth, it is possible that the secosterols were misidentified to begin with. We are investigating this further by UPLC-MS to confirm or deny Wentworth's original proposal. In essence, this pathway to the secosterols would be much more plausible than the involvement of ozone *in vivo*.

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PPA34

Source emission-related modulation of biological pathways relevant to vascular changes in healthy voulunteers

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Epidemiological investigations have shown consistent increase in cardiovascular events in association with short and long term exposure to ambient air pollution. Air pollutants are mixtures of various gases and particulate matter with varying physicochemical characteristics and thus toxicity. The objective of this work is to investigate biological changes in plasma and saliva of healthy volunteers who spent five consecutive days near a steel plant and at a site several kilometers away from the plant to gain mechanistic understanding of source-emission related changes. Plasma and saliva markers of oxidative stress, inflammation and endothelial injury were analysed by multiplex protein-array, HPLC-Fluorescence, EIA and ELISA methods. The biochemical, physiological, and air pollution data were analysed for associations using backward stepwise and best subsets regression analyses. Our results indicated that plasma big endothelin-1 levels were positively associated with systolic blood pressure ($p=0.009$) and were predicted by weekly averages of pollutants NO_2 ($p=0.010$) and SO_2 ($p=0.017$) collected at each site. Also, diastolic blood pressure was predicted ($r^2=0.370$, $p<0.001$) by salivary endothelin ET1-31, plasma IL-8 and 8-isoPGF2α (lipid oxidation marker). Both endothelins and 8-isoPGF2α are known to exhibit vasoregulatory properties. Furthermore, SO_2 levels were almost quadrupled at the steel mill site compared to the site well removed from there. Our results suggest that source emission can impact vascular homeostasis.

PPA35

Northern contaminants reduce serum and pancreatic insulin levels due to β cell destruction

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Rates of obesity and diabetes mellitus of Arctic populations are increasing due to multiple reasons including a departure from traditional lifestyles and chronic and excessive consumption alcohol. These populations are also simultaneously exposed to a variety of anthropogenic contaminants which can contribute to development of various metabolic disorders. Our group has previously shown that a Northern Contaminant Mixture (NCM), containing 22 most abundant organic and inorganic contaminants found in the blood of Canadian Arctic populations, induces endothelial cell dysfunction and contributes to the development of non-alcoholic fatty liver disease. In order to determine if Northern contaminants have a direct effect on pancreas function and physiology, lean and obese JCR rats were orally treated with the NCM or vehicle at low and high doses. These treatments were done in the presence and absence of ethanol to assess further involvement of increased alcohol consumption in impairing metabolic function.

NCM treatment reduced circulating and pancreatic insulin levels as a result of direct pancreatic injury with β cell loss observed by immunohistochemical analysis. Studies conducted with cultured insulin-secreting Min6 cells showed that the NCM inhibited insulin release and induced cell death through oxidative stress and mitochondrial dysfunction. Furthermore, we identified that 2,3,4,6-tetrabromophenol, one of the components of the NCM, attenuates beta cell function as it rapidly inhibits insulin release from Min6 cells following a 10 minute exposure. The results strongly suggest that these contaminants may contribute to pancreatic dysfunction and diabetes in some of the highly exposed Northern Canadian inhabitants.

PPA36

Effects of Polyphenolic Metabolites on H_2O_2-induced Inflammation in Human Calu-3 Airway Epithelial Cells

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Environmental agents such as smoke, pollution, and chemical inhalants can cause respiratory injury via cascades of intracellular processes leading to inflammation and oxidative stress. Dietary polyphenols have shown to exert anti-inflammatory effects that could be protective against lung damage; however, despite an abundance of research on parent polyphenolic compounds, research on their metabolites generated post-intestinal and hepatic metabolism is limited. A polyphenol-rich potato extract, ≈88% of the polyphenol content is chlorogenic acid (CGA), was subjected to digestion using a computer controlled dynamic human gastrointestinal (GI) model. Colonic digests of the GI model underwent the bio-transformative and absorptive processes of CaCo-2/HepG2 co-cultures to mimic human intestinal and hepatic first pass metabolism. 3-Phenylpropionic acid (PPA) and benzoic acid (BA) were the only metabolites detectable by electrospray time-of-flight mass spectrometry after CaCo-2/HepG2 metabolism of the colonic digests. The anti-inflammatory effects of the parent compound, CGA, and its metabolites (PPA and BA) on respiratory epithelial cells (Calu-3) were tested against H_2O_2-induced oxidative stress. Cells were subjected to a 24 h incubation with physiologic (0.5, 1, 2, 4 µM) or supraphysiologic concentrations (100 µM) of polyphenols or glutathione ester (GSH-E) at 0.3 mM after which they were exposed to 4 h of 1 mM H_2O_2 and incubated for an additional 24 h period. In recovery experiments, H_2O_2 exposure preceded the treatment. H_2O_2 exposure caused a significant increase in the release of IL-8 by 947±154% (P less than 0.05). GSH-E, when administered before H_2O_2, decreased the H_2O_2-mediated release of the pro-inflammatory cytokine IL-8 by 25±2% (P less than 0.05). Administration of CGA at 4 µM, prior to H_2O_2 treatment, was protective by inhibiting IL-8 stimulation via H_2O_2 by 133±32 pg/ml (P less than 0.001), a 22% decrease. A major decrease in cell viability (14-45%) (P less than 0.05) was detected for all polyphenols at the 100 µM dose, both in the absence and presence of H_2O_2. Cell viability also decreased by 30% ($P < 0.05$) at the 4 µM dose of BA in the presence of H_2O_2. Overall, this project indicates that physiologic concentrations of chlorogenic acid but not its metabolites can provide anti-inflammatory pulmonary protection induced by oxidative stress.

PPA37

Oxidative stress and mitochondrial damage leading to apoptosis as the mechanisms of cadmium telluride quantum dot nanoparticle toxicity

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Quantum dots (QDs) are a class of engineered nanoparticles (NPs) that range from 2 to 100 nm in diameter. Due to their unique optical and electronic properties, QDs have been used in different technologies including solar cells, light emitting devices, quantum computing and other applications. These NPs also hold great promise as an important tool in medical imaging, cancer detection, and targeted drug delivery. While the usefulness and applications of QDs continue to expand, there have been concerns about their toxicity. The current research investigates the underlying mechanisms of toxicity of cadmium telluride QDs (CdTe-QDs) in human hepatocellular carcinoma HepG2 cells. The study demonstrated that exposure to CdTe-QDs resulted in increased generation of reactive oxygen species, changes in select antioxidant defense systems and effects on mitochondrial structure and function. Furthermore, CdTe-QD treatments resulted in activation of extrinsic and intrinsic apoptotic pathways leading to cell death. Our results also showed that the effects of CdTe-QDs were similar or greater to those of $CdCl_2$ at equivalent concentrations of cadmium, suggesting that the toxic effects of CdTe-QDs were not solely due to cadmium released from the NPs. The study provides insight on the toxicity of CdTe-QDs which is important for understanding potential hazards associated with future biomedical applications.

PPA38

Evidence of oxidative stress in a murine model of Hereditary Tyrosinemia type I after treatment withdrawal

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Hereditary Tyrosinemia type 1 (HT1) is a severe metabolic liver disease caused by genetic defects of fumarylacetoacetate hydrolase (FAH), the last enzyme involved in the tyrosine catabolic pathway. The lack of expression of this enzyme results in severe hepatic dysfunction caused by the upstream accumulation of three toxic metabolites: fumarylacetoacetate (FAA), maleylacetoacetate (MAA) and succinylacetone (SA). Despite the daily intake of NTBC (2-[2-nitro-4-(trifluoromethyl)benzoyl]cyclohexane-1,3-dione) as effective treatment to stop the progression of liver damage, risk of developing hepatocellular carcinoma (HCC) remains high in HT1 patients. As previously demonstrated, FAA has been shown to deplete intracellular glutathione (GSH) by forming stable adducts which leads to lower GSH cellular concentrations thus imbalancing the redox state of the cells. Accordingly, it has been reported that GSH-MEE/vitamin C treatment started during pregnancy is as effective as NTBC, in rescuing fah-/- newborn mice from death during the first 24h. GSH allows an effective neutralization of reactive oxygen species (ROS) by enhancing the activity of antioxidant enzymes such as glutathione peroxidase (GPx) and glutathione reductase (GR). To measure the involvement of oxidative stress in the progression of HT1, Western blot analysis were performed on untreated fah-/- mice at different stages of the disease. A change of NF-E2-related transcription

factor 2 (Nrf2), a key transcriptional regulator in driving antioxidant gene expression, was observed shortly after NTBC removal. Changes in markers of stress such as NAD(P)H quinine oxidoreductase 1 (NQO-1) and heme oxygenase 1 (HO-1) were also correlated with disease progression. Furthermore, our results show an increased expression of HSPA1A and HSPB1, two heat shock proteins (HSPs) involved in stress defense. All together, these data demonstrate the importance of oxidative stress in HT1 phenotype. (Supported by the Canadian Institutes of Health Research and GO Foundation)

PPA39
Mechanisms of induction of apoptotic cell death by heat shock
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Hyperthermia is used in cancer treatment in combination with radiotherapy and/or chemotherapy. Elevated temperatures are able to kill cancer cells and decrease the size of tumors. Despite promising results from clinical trials, the mechanisms involved are not understood. It is in this perspective that the expression of proteins involved in cellular defenses was studied to better understand mechanisms involved in the toxicity of hyperthermia (42-43°C) in cancer cells. We also studied moderate thermaltolerance (40°C), which is an adaptive response that allows cells to continue normal function when exposed to toxic stresses. In particular, our interest concerned peroxiredoxins (Prxs), which are proteins playing a role as antioxidants in the detoxification of peroxides. Peroxiredoxins help to protect proteins against the oxidation of thiol groups, which are critical for the biochemical function of a majority of proteins. In addition, glucose metabolism is important for generation of energy (ATP). Glucose in blood enters cells by GLUT transporter proteins. Furthermore, NADPH generated by glucose metabolism is essential to maintain the intracellular antioxidant glutathione in its reduced form. The critical enzyme in glucose metabolism is glucose 6-phosphate dehydrogenase (G6PD). Heat shock at 42 and 43°C increased the expression of the proteins PrxII , PrxIII and G6PD, compared to the control at 37°C, in HeLa cells. The protein expression of PrxII and PrxIII also increased during thermotolerance at 40°C. These results will contribute to improving our knowledge on the adaptive survival response under conditions of toxic stress, and on the mechanisms of toxicity and cell death induced by hyperthermia.

PPA40
Evaluation of Benzaldehyde and Related Compounds-Induced Protein Carbonyl Formation in a Cell Free System
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Aromatic aldehydes have been found in car emissions and may contribute to protein carbonylation. Aromatic aldehydes are also used as spices and as flavoring and aroma agents to foods and beverages. Benzaldehyde is a major component of essential oils of the seeds and kernels of bitter almonds, apricots, peaches, plums and cherries. Protein carbonylation may also play pivotal role in etiology or/and progression of life-threatening diseases, i.e. neurodegenerative, aging and cancer by increasing reactive carbonyl stress (RCS). We tested benzaldehyde and 17 other benzaldehyde-like compounds for protein carbonylation formation effectiveness using the 2,4-dinitrophenylhydrazine (DNPH) assay and the protein carbonylation of bovine serum albumin (BSA) at different concentrations. In particular we investigated how the addition of hydroxyl, bromine, chlorine, and methyl functional groups at the ortho, meta and para positions on the benzyl ring of benzaldehyde affects the

production of carbonyls. Benzaldehyde and substituted benzaldehydes produced a significantly higher concentration of protein carbonyls. At the para position, the addition of a hydroxyl, bromine, chlorine, or methyl functional group to benzaldehyde molecule markedly increased its protein-carbonyl formation. In the meta position, the addition of chlorine increased the extent and rate of the addition of carbonyls on proteins. Adding a bromine functional group only increased the rate but not to the extent of the addition. 3-hydroxybenzaldehyde and 3-tolualdehyde were slower at protein carbonylation. Hydroxyl, bromine, chlorine, and methyl groups in the ortho position showed a significant decrease in protein carbonyl formation at all concentrations and time points. The rates of added carbonyl functional groups to BSA of cinnamaldehyde, vanillin, and cuminaldehyde were similar to that of benzaldehyde at 0 hr and 1 hr time points, but decreased at the 2 hr and 3 hr time points. The highest protein carbonylation rate was: 4-chlorobenzaldehyde> 4-hydroxybenzaldehyde> 4-bromobenzaldehyde> 3-chlorobenzaldehyde> benzaldehyde> 3-bromobenzaldehyde> 4-tolualdehyde> cinnamaldehyde> vanillin> cuminaldehyde> 3-tolualdehyde> 3-hydroxybenzaldehyde. This study therefore questions the biosafety of some benzaldehydes particularly 4-chlorobenzaldehyde and 4-hydroxybenzaldehyde. However, thiol group containing compounds e.g., glutathione, N-acetylcysteine, mesna (2-mercaptoethanesulfonate) and the carbonyl scavenger, aminoguanidine markedly prevented protein-carbonyl formation induced by aromatic aldehydes.

PPA41

Parkinsonian features in aging GFAP.HMOX1 transgenic mice overexpressing human HO-1 in the astroglial compartment

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Objective: To determine whether mid-to-late life overexpression of glial heme oxygenase-1 (HO-1), a stress protein induced in the Parkinson disease (PD) substantia nigra, recapitulates neurochemical, neuropathological and behavioural features of the disease in an animal model. Methods: Motoric behaviour, basal ganglia neurotransmitter levels and neuropathological markers were ascertained in conditional GFAP.HMOX1 transgenic mice expressing human HO-1 (HMOX1) in the astrocytic compartment from 8.5 to 19 months of age. Results: HMOX1 expression was documented in astrocytes, ependymocytes and tanycytes. Relative to wild-type controls, the GFAP.HMOX1 mice exhibited impaired motor coordination (rotarod test), striatal dopamine deficiency and augmented substantia nigra GABA concentrations (HPLC-EC), pathological brain iron deposition (DAB-Perls stain), increased neuronal and glial MnSOD protein (mitochondrial OS marker; IHC), and increased ubiquitin staining in astrocytes and tyrosine hydroxylase-positive (dopaminergic) neurons (IHC). Impaired motor performance did not occur in transgenic mice overexpressing glial HMOX1 between 1.5 and 12 months of age, attesting to the important role of brain aging in this model. Conclusions: Corroborating and extending our earlier in vitro findings to the intact brain, over-expression of astrocytic HMOX1 in mice between 8.5 and 19 months of age promotes several behavioural, neurochemical and neuropathological features of idiopathic PD. Curtailment of glial HO-1 hyperactivity by pharmacological or other means may afford neuroprotection in PD and other aging-related neurodegenerative disorders.

PPA42

Cholesterol Autoxidation Revisited

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While our understanding of the mechanisms of polyunsaturated fatty acid oxidation has come a long way since the mid 1980s,1,2 cholesterol oxidation has received comparatively little attention. This is somewhat surprising given that it is the most vilified of lipids, and comprises approximately 20 percent of mammalian cell membranes, and up to 50 percent in lipid rafts. The general consensus following work in the 1960s through the 1980s is that cholesterol autoxidation leads to one primary product: the 7-hydroperoxide.3 Cholesterol oxidation products are believed to have pathological roles. Of most recent interest have been the so-called atheronals, the two carbonyl-containing cholesterol oxidation products first identified as ozonolysis products of cholesterol, that have since been identified in extracts of arterial plaque and brain tissue,4 and a debate has since ensued over their origin. Our group has demonstrated that the atheronals can be formed from Hock fragmentation of cholesterol 5-hydroperoxide, the product of singlet oxygen's reaction with cholesterol. We have since wondered if this cholesterol 5-hydroperoxide can also be formed from cholesterol autoxidation, and that it has somehow been missed over the years. To shed further light on the mechanism of cholesterol autoxidation, we have investigated the various possible pathways using computational methods. These studies suggest that while the 7-hydroperoxide is predicted to be the favored product, the 5-hydroperoxide should still arise under kinetically-controlled conditions. To complement these results, we have undertaken experimental studies of cholesterol autoxidation under various conditions, in an effort to determine under which conditions the cholesterol 5-hydroperoxide can be formed.

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PPA43

Mutant Huntingtin mediated repression of antioxidant gene expression is rescued by a novel Nrf2 activating agent

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Mitochondrial dysfunction and elevated reactive oxygen species (ROS) levels are strongly implicated in various neurodegenerative disorders, including Huntington's disease (HD). Expression of the mutant Huntingtin protein (mHtt) containing an expanded polyglutamine repeat is associated with oxidative stress and toxicity in striatal neurons. We previously demonstrated that overexpression of mHtt in PC12 cells leads to elevated ROS production and a concomitant decrease in expression of the antioxidant protein peroxiredoxin1 (Prx1). Interestingly, treatment with the compound dimercaptopropanol (DMP) prevents mHtt-mediated inhibition of antioxidant gene expression and neurotoxicity. Nuclear factor erythroid 2-related factor 2 (Nrf2) is a transcription factor responsible for regulating expression of a diverse array of antioxidant genes under the control of cis-acting antioxidant response elements (AREs), including Prx1. Nrf2 is maintained at very low levels by its negative regulator Kelch-Like ECH-associated Protein 1 (Keap1), which facilitates the ubiquitination and subsequent degradation of Nrf2 by the proteasome. Post-translational modification of Keap1 and/or Nrf2 by electrophiles and oxidants disrupts the Keap1-Nrf2 interaction, resulting in the stabilization and nuclear translocation of Nrf2. There is currently great interest in

identifying Nrf2 activating compounds for the treatment of neurodegenerative diseases. Here we demonstrate that mHtt prevents Nrf2 nuclear translocation and activation of antioxidant enzyme expression in PC12 cells and in an immortalized striatal cell line (STHdhQ111). In contrast, DMP exposure decreases the levels of Keap1, thereby allowing activation of Nrf2 even in the presence of mHtt. Preliminary findings indicate that DMP promotes the degradation of Keap1, possibly via an autophagic/lysosomal process. The identification of DMP as a neuroprotective agent that facilitates the degradation of Keap1 and promotes Nrf2 activation is highly novel and suggests that alternative modes of Nrf2 activation exist. In addition, DMP, also known as British anti-Lewisite (BAL), was shown to attenuate disease progression in a long term study of two HD patients conducted in 1955. The current study highlights previously unknown intracellular targets of DMP and indicates that this FDA approved compound may have relevance for the treatment of HD and other neurodegenerative disorders.

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Modulation of Nrf1 by endoplasmic reticulum stress and the unfolded protein response

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Nrf2 (nuclear factor-erythroid 2 p45 subunit related factor 2) plays a key role in the expression of stress-related genes in response to xenobiotic, electrophilic, and oxidative stress. The mechanism modulating Nrf2 translocation to the nucleus involves dissociation from Keap1 following oxidative or electrophilic stress in the cytosol or mitochondria. Nrf1, a protein homologous to Nrf2, is also required for expression of stress-related genes following oxidative or electrophilic stress. However, some key features separate Nrf1 from Nrf2 including its localization to the endoplasmic reticulum (ER) and that Keap1 is not required for its regulation. More importantly, less is known about the modulation of Nrf1. Due to its ER localization we hypothesized that Nrf1 was sensitive to activation and stabilization by the unfolded protein response (UPR). HEK293t cells were grown to confluency and treated with thapsigargin (THP; 0-10 nM) and tunicamycin (TUN; 0-10 μg/mL), drugs known to induce ER stress and UPR. Cells exposed to antimycin A (AA; 0-5 μM), a Complex III inhibitor and mitochondrial poison, was also used to determine if mitochondrial stress could lead to Nrf1 stabilization. THP and TUN stabilized Bip/Grp78, a protein involved in the UPR response. AA treatment did not induce Bip/Grp78 stabilization. THP and TUN did not induce any increases in cellular ROS and cell death at all doses whereas AA treatment induced a dose-dependent increase in cellular ROS followed by an induction of cell death at the highest dose. AA treatment also induced an increase in Nrf2 levels which was not observed in cells treated with various amounts of THP or TUN. However, a dose dependent increase in the active form of Nrf1 (P95) was observed in cells treated with THP or TUN with TUN having a more potent effect. In addition, a trend for decrease in the Nrf1 degradation fragment (P23) was also observed in cells exposed to THP. No changes in P95 were observed in AA-treated cells. Taken together, our results indicate that two separate pathways are required for the stabilization of Nrf1 and Nrf2 respectively. Both pathways are responsive to organelle stress with Nrf1 modulated by ER stress and Nrf2 induced by mitochondrial stress.

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