Session 1: A Tribute to Balz Frei

Chair: Tory M. Hagen, PhD, Linus Pauling Institute, Oregon State University, Corvallis, OR

Wednesday, September 13th
9:00 - 10:30 AM
Cardiovascular disease remains the number one source of morbidity and mortality in the developed world. A key control point for the development of atherosclerosis and its clinical sequelae is vascular function that resists thrombosis, inflammation, and vasospasm. In collaboration with Balz Frei and Joseph Vita, we embarked on a research program to investigate how the redox environment of the endothelium dictates vascular function. We found that normal vascular homeostasis was dependent upon both the extracellular presence of reactive oxygen species and the intracellular redox state, dictated largely by small molecule antioxidants. Using combinations of studies in cell culture and human subjects, the roles of ascorbic acid, glutathione, and tetrahydrobiopterin was elucidated. More recently, this program has probed the involvement of mitochondria and stress-activated signaling pathways in the control of vascular function.
3,3'-Diindolylmethane (DIM) is a biologically active derivative of indole-3-carbinol, an anticancer agent from cruciferous vegetables. Early DIM research focused on xenobiotic metabolism changes linked to the inhibition of mammary and liver tumorogenesis [Wattenberg & Loub, Cancer Res 1978; Bradfield & Bjeldanes, J Toxicol Environ Health 1987; Dashwood et al., Cancer Res 1994]. Subsequent work implicated DIM in signaling pathways regulating cell division, apoptosis, and angiogenesis [reviewed by Licznerska & Baer-Dubowska, Adv Exp Med Biol 2016]. Research with DIM moved into the epigenetic realm with evidence for changes in histone deacetylase (HDAC) enzymes, chromatin modifiers, genome-wide DNA methylation, and miRNA networks [Li et al., Cancer Res 2010; Beaver et al., Toxicol Appl Pharmacol 2012; Wong et al., PLoS One 2014; Palomera-Sanchez et al., J Nutr Biochem 2017; Hanieh, Mol Cancer 2015]. Parallel investigations focused on sulforaphane (SFN), another anticancer agent from cruciferous vegetables [Zhang et al., PNAS 1992]. As an electrophile, SFN targets Keap1 to release Nrf2 into the nucleus [Yang et al., Semin Oncol 2016]. Among the Nrf2 target genes are enzymes that convert SFN into water-soluble metabolites that inhibit HDAC activity [Myzak et al., Cancer Res 2004]. SFN-cysteine and SFN-N-acetylcysteine metabolites target the HDAC3-SMaRT corepressor complex for inhibition/turnover, altering the acetylation status of both histone and non-histone proteins [Rajendran et al., Mol Cancer 2011; Rajendran et al., Epigenetics 2013; Rajendran et al., Clin Epigenetics 2015]. Transcriptomics (RNA-seq) also defined SFN-induced changes in noncoding RNAs, including repression of IncRNA LINC01116 in prostate cancer cells [Beaver et al., J Nutr Biochem 2017], and upregulation of a novel functional pseudogene, NMRAL2P, in colon cancer cells [Johnson et al., Mol Nutr Food Res 2017]. Clinical validation of these mechanistic targets is now ongoing in human volunteers.
Roles of Bioactives in Redox Regulation

Helmut Sies, MD

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A set of four principles underlying redox biology has been denoted as the "Redox Code" (1). First, redox regulation utilizes the NAD⁺ and NADP⁺ systems in catabolic and anabolic metabolic pathways, respectively, and small molecular mass compounds, termed bioactives, play a role in pool size and certain functions, e.g. as precursors of substrate reacting with SIRT3. Second, thiol redox transitions are used in redox signaling via the sulfenome; again, bioactives can modulate by addressing molecular redox switches such as Nrf2/Keap1. Third, the hydrogen peroxide tone in oxidative eustress (2) is controlled, in part, by links with bioactives. Fourth, the global redox network involves set-points which are under the influence of bioactives. Examples for these four areas of active research will be given: nicotinamide, sulforaphane, selenocompounds, polyphenols. Bioactives are functional in dampening postprandial oxidative stress.

A brief comment on the conundrum of terminology regarding bioactives, nonessential nutrients, micronutrients, antioxidants, vitamins and their relation to oxidative stress (3) will conclude the presentation.

Session 2: Dietary Components and the Microbiome

Chair: Adrian "Fritz" Gombart, PhD, Linus Pauling Institute, Oregon State University, Corvallis, OR

Wednesday, September 13th
11:00 AM - 12:00 PM
Humans have evolved with dense microbial populations that colonize their gastrointestinal tract and are integral to our biology, for example through the provision of signals that aid the development of the immune system. Increasing evidence indicates that modern lifestyle, and specifically a western diet, has led both to a substantial depletion of the human gut microbiome, as well as differences in the bacterial metabolism. Low-fiber diets provide insufficient nutrients for the gut microbes, leading not only to the loss of species reliant on these substrates but also to a reduction in the production of fermentation end-products with important physiological and immunological functions. In other words, by shifting to a diet that is fundamentally different to the diet under which the human-microbiome interrelationship evolved, we might have disrupted this symbiosis, reducing or removing the evolutionary-routed benefits provided by the microbes. The notion that this process is implicated in the rampant increase of chronic diseases provides a strong incentive to consider attempts to conserve and potentially restore the gut microbiome.
Poorly absorbed polyphenols are associated with metabolic resilience raising questions about their mechanisms of action. C57BL/6j mice fed high-fat diet (HFD) supplemented with 1% grape polyphenols (GP) for 12 weeks showed attenuated metabolic syndrome symptoms and changes in gut gene expression consistent with metabolic resilience. Mice also developed a bloom in Akkermansia muciniphila, a mucin dwelling gut microbe associated with metabolic health. To better understand the dynamics of these GP-induced changes we investigated the timing of the A. muciniphila bloom and the responsible class of GP. In two 14-day time course studies mice were fed HFD, low-fat diet (LFD), or formulations supplemented with 1% GP (HFD-GP, LFD-GP). Mice fed HFD-GP for two weeks showed significantly improved oral glucose tolerance (OGT) compared to control, while LFD and LFD-GP groups displayed similar OGT. In a separate study, mice gavaged with GP extract (GPE) or grape proanthocyanidins (PAC), in each case delivering 360 mg PAC/kg body weight, showed increased fecal and cecal A. muciniphila. Initial abundance of intestinal A. muciniphila determined the rate of GPE- or PAC-induced bloom. A. muciniphila growth in vitro was inhibited by exposure to GPE or PAC. Our data suggest that grape PAC are sufficient to induce a bloom of A. muciniphila, which can occur independently of specific changes in intestinal gene expression. In vitro inhibition of A. muciniphila by GPE or PAC suggests that, rather than directly promoting growth, PAC alter the gut microbiota providing A. muciniphila with a selective growth advantage that may benefit host metabolic health.
Session 3: Lipid Metabolism

Chair: Fred Stevens, PhD, Linus Pauling Institute, Oregon State University, Corvallis, OR

Wednesday, September 13th
1:30 - 3:00 PM
**Cardiac Benefits of Medium-Chain Triglyceride Supplements: Odd versus Even**

Melanie Gillingham, PhD, RD

*Associate Professor, Department of Molecular and Medical Genetics*  
*Graduate Programs in Human Nutrition*  
*Oregon Health & Science University, Portland, OR*

**Background:** Unlike long-chain triglycerides (LCT), Medium-chain triglyceride (MCT) supplements are reported to be absorbed via the portal vein, preferentially oxidized in liver and peripheral tissues, and associated with increased energy expenditure. Clinically MCT has been used in patients with fat malabsorption syndromes and in patients with long-chain fatty acid oxidation disorders (LC-FAODs) to bypass the long-chain fatty acid oxidation pathway. Traditional MCT is typically composed of C8, C10 and some C12 fatty acids. Observational reports suggest that supplementation with odd chain MCT such as triglycerides composed of C7 fatty acids that increase citric acid cycle intermediates via anaplerosis may have therapeutic advantages over traditional MCT for the treatment of LC-FAODs but controlled trials have not been reported. The goal of our study was to compare the effects of triheptanoin (C7), an anaplerotic seven-carbon fatty acid triglyceride, to trioctanoin (C8), an eight-carbon fatty acid triglyceride, in patients with LC-FAODs.

**Methods:** A double blinded, randomized controlled trial of thirty-two subjects with LC-FAODs (carnitine palmitoyltransferase-2, very long-chain acyl-CoA dehydrogenase, trifunctional protein or long-chain 3-hydroxy acyl-CoA dehydrogenase deficiencies) who were randomly assigned a diet containing 20% of their total daily energy from either C7 or C8 for 4 months was conducted. Primary outcomes included changes in total energy expenditure (TEE), cardiac function by echocardiogram, exercise tolerance, and phosphocreatine recovery following acute exercise. Secondary outcomes included body composition, blood biomarkers, and adverse events, including incidence of rhabdomyolysis.

**Results:** Patients in the C7 group increased left ventricular (LV) ejection fraction by 7.4% (p=0.046) while experiencing a 20% (p=0.041) decrease in LV wall mass on their resting echocardiogram. They also required a lower heart rate for the same amount of work during a moderate-intensity exercise stress test when compared to patients taking C8. There was no difference in TEE, phosphocreatine recovery, body composition, incidence of rhabdomyolysis, or any secondary outcome measures between the groups.

**Conclusions:** C7 improved LV ejection fraction and reduced LV mass at rest, as well as lowering heart rate during exercise among patients with LC-FAODs. Triheptanoin improved the cardiorespiratory fitness of patients over traditional MCT.
Phenotypic Flexibility: A New Way to Quantify the Effects of Food and Nutrition on Health

Suzan Wopereis, PhD

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Netherlands Organization for Applied Scientific Research (TNO)
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The focus is shifting from disease prevention to health optimization, but nutrition science has struggled to demonstrate specific health benefits related to diet or dietary ingredients. That’s partly because it’s difficult to measure health status changes in (apparently) healthy human subjects. Health can be seen as a dynamic state in which the ability to adapt can be an indicator of health status.

In daily life, people cope continuously and subconsciously with changes in their environment, including the intake of suboptimal foods or levels of physical exercise. Their ability to adapt can act as an indicator for maintenance or improvement of physiological function. The term ‘phenotypic flexibility’ expresses the cumulative ability of overarching physiological processes (e.g. metabolism, inflammation, oxidation) to return to homeostatic levels after short term perturbations.

This presentation will outline research, focused on assessing objective approaches for quantifying health in diet-related context, and describe the results from studies where we have used phenotypic flexibility as a readout with special focus on metabolic and inflammatory responses.
Phospholipid Oxygenation Products and their Role as Potential Proximate Signals of Ferroptotic Death

Valerian E. Kagan, PhD, DSc

Professor and Vice Chairman,
Department of Environmental and Occupational Health
University of Pittsburgh, Pittsburgh, PA

The reliability of biological processes is achieved, to a large extent, through the initial generation of excessive amounts of biological material, including organelles and cells, which upon successful completion of their specific functions become unnecessary. Removal of the unnecessary in a timely manner is essential to prevent the unnecessary from becoming undesired or even harmful. This principle applies to the elimination of damaged unnecessary organelles and cells. While mitophagy is a pro-survival rescue pathway, the continued accumulation of mitochondrial impairments and the insufficiency and/or failure of repair mechanisms may necessitate the elimination of the entire cell through the activation of the apoptotic program. This requires a process of externalization of a mitochondria-specific phospholipid, cardiolipin (CL) followed by oxidation of its polyunsaturated acyl chain(s). The oxidation reaction is catalyzed by intramembrane space hemoprotein, cytochrome c that forms a high affinity complex with cardiolipin. There is thus, a transformative structural shift for cytochrome c from being an electron carrier shuttling electrons between respiratory complexes III and IV while located on the outer surface of the IMM, to a CL-specific peroxidase function in apoptosis. Another type of programmed cell death is a newly discovered ferroptosis, a form of regulated necrotic cell death controlled by glutathione peroxidase 4 (GPX4). Enigmatic lipid peroxidation products are the proximate executioners of ferroptosis. We discovered that ferroptosis involves a highly organized oxygenation center, wherein oxidation in endoplasmic-ribosomal-associated compartments occurs in only one class of phospholipids (phosphatidylethanolamines (PEs)) and is specific toward two fatty acyls—arachidonoyl (AA) and adrenoyl (AdA). Suppression of AA or AdA esterification into PE by genetic or pharmacological inhibition of acyl-CoA synthase 4 (ACSL4) acts as a specific antiferroptotic rescue pathway. 15-Lipoxygenase (15-LOX) generates doubly and triply-oxygenated (15-hydroperoxy)-diacylated PE species, which act as death signals.
Session 4: Update on Vitamin E
Chair: Maret Traber, PhD, Linus Pauling Institute, Oregon State University, Corvallis, OR

Wednesday, September 13th
3:30 - 5:30 PM
Vitamin E Regulation of the Development of Allergies and Allergic Asthma Immunopathogenesis

Joan Cook-Mills, PhD

Professor of Medicine,
Division of Allergy-Immunology
Northwestern University, Chicago, IL

Background: Allergic disease and asthma result from complex interactions of environmental and genetic factors. The World Health Organization reported increased prevalence of asthma from 1950 to the present. The marked rise in rates of asthma over a few decades and the differences in rates among countries and in migrating populations suggest an important role of the local environment, such as diet, in development of asthma. One of the environmental changes over the past 40 years has been an increase in the vitamin E isoform d-γ-tocopherol (γT) in the diet and in infant formulas that contain soybean oil which is rich in γT.

Results: We demonstrated that the vitamin E isoform d-α-tocopherol (αT) blocks allergic responses in adult mice. In contrast, a 5-fold increase in γT in tissues increases eosinophilic allergic lung inflammation (175%) and airway hyperresponsiveness in adult mice. In mechanistic studies of signals for recruitment of eosinophils to the site of allergic inflammation, we demonstrated that αT and γT bind the C1a regulatory domain of protein kinase Cα and compete with diacylglycerol and retinol for binding to the C1a domain. Moreover, αT and γT functioned as an antagonist and agonist of PKCα, respectively. To translate our findings for αT and γT to humans, we demonstrated that adults with high γT and low αT in plasma had increased odds of lower lung function by 4 fold in 8 years. In another clinical study, we demonstrated that a 5-fold higher plasma γT level associates with lower lung spirometry (10 to 17% decrease in FEV1) in adults by age 21, suggesting that early in life, tocopherol isoforms may regulate development and lung responses to environmental exposures such as allergens. Allergies often develop early in life and, importantly, offspring of allergic mothers have increased responsiveness to allergen challenge. In a mouse model, we demonstrated that in the fetal liver of allergic mothers, there is an increase in distinct subsets of CD11b+CD11c+ inflammatory dendritic cells and cytokines that regulate development of allergic responses. This increase in dendritic cells and cytokines was blocked by supplementation of the mother with αT but, elevated by supplementation of the mother with γT.

Conclusions: αT and γT have opposing regulatory functions in allergic responses and in development of allergic disease. The opposing regulatory functions of αT and γT impact interpretations of previous studies with disparities of vitamin E effects on inflammation in basic and clinical research. Moreover, our data have important implications for tocopherol regulation of the development of allergic disease in offspring and future generations.
α-Tocopherol Bioavailability is Lower in Adults with Metabolic Syndrome

Richard S. Bruno, PhD, RD

Professor, Human Nutrition Program
The Ohio State University, Columbus, OH

Dietary recommendations for α-tocopherol, the only essential form of vitamin E, are challenging to meet from food alone. Indeed, >92% of Americans fail to meet recommendations, and α-tocopherol status is likely further compromised by inflammatory responses occurring in metabolic syndrome (MetS). It is therefore important to establish the extent to which MetS increases dietary α-tocopherol requirements due to underlying inflammation that likely impairs α-tocopherol trafficking and metabolism along the gut-liver axis. Our approach entailed a randomized cross-over study in MetS and age- and gender-matched healthy adults in which they ingested encapsulated deuterium-labeled RRR-α-tocopherol (15 mg) with dairy milk beverages containing 0-8 g fat. Pharmacokinetics of labeled α-tocopherol and its physiologic metabolite α-CEHC (carboxyethyl-hydroxychromanol) were then assessed for 72 h using LC-MS. MetS adults had increased circulating oxidized LDL and pro-inflammatory cytokines in association with metabolic endotoxemia. Plasma α-tocopherol absorption, C_{max}, and bioavailability (AUC_{0-72 h}) were significantly lower in MetS adults regardless of dairy milk fat content. Their isolated chylomicrons and VLDL also had lower α-tocopherol enrichment, suggesting impaired intestinal and hepatic trafficking of α-tocopherol. MetS adults also had lower circulating and urinary α-CEHC, suggesting inadequate α-tocopherol status to activate P450-mediated metabolism of α-tocopherol. Pharmacokinetic parameters of both α-tocopherol and α-CEHC were correlated with inflammatory responses. Thus, MetS adults have higher dietary requirements to offset inflammatory stress responses that limit α-tocopherol bioavailability by decreasing intestinal absorption and hepatic secretion. This presentation will therefore discuss the potential mechanisms by which MetS impairs α-tocopherol status, and implications for achieving optimal health.
Session 5: Bioactives and Cancer Prevention

Chair: Arup Indra, PhD, Linus Pauling Institute, Oregon State University, Corvallis, OR

Thursday, September 14th
8:00 - 9:30 AM
Cruciferous vegetables are a rich source of dietary indoles. Indole-3-carbinol (I3C), and 3,3’-diindolylmethane (DIM) are formed from glucobrassicin myrosinase found both in the plant and intestinal bacteria. I3C is unstable in the G.I. forming acid condensation products including DIM. We have studied cancer chemoprevention by dietary indoles in various models. Early work, led by Dr. George Bailey, established rainbow trout as a model for I3C chemoprevention of cancer. In the last decade we found supplementing diet of pregnant mice with I3C reduced offspring mortality from polycyclic aromatic hydrocarbon (PAH)-induced T-cell acute lymphoblastic leukemia (T-ALL). Immuno-compromised mice, injected with human T-ALL cells, had marked reduction in tumor growth when fed DIM. Studies in vitro with T-ALL cells displayed marked reductions in proliferation and viability along with enhanced apoptosis. We are now testing zebrafish embryos as a xenograft model for human T-ALL. Also, utilizing accelerator mass spectrometry ($^{14}$C at atto-zeptomole range) we hope to test DIM and Brussels sprouts in enhancing metabolism and excretion of $^{14}$C-PAHs when humans are “micro-dosed” at levels at or below daily exposures.

Early studies of cancer chemoprevention by indoles focused on “blocking” mechanisms. I3C and DIM inhibit enzymes that bioactivate carcinogens and induce detoxication enzymes thus reducing covalent DNA binding. I3C acid condensation products are aryl hydrocarbon receptor (AHR) ligands. AHR regulates many important pathways including cell cycle, apoptosis, autophagy, angiogenesis, oxidative stress and chronic inflammation. Currently, we are assessing epigenome modulation and cancer chemoprevention. I3C and DIM inhibit histone deacetylases (HDACs), alter chromatin structure, expression of select microRNAs and methylation status of promoter regions of genes involved in carcinogenesis. Epidemiology studies consistently show an inverse correlation between cruciferous vegetable intake and cancer incidence. However, clinical intervention trials have been somewhat disappointing. The potential for indole supplements in chemoprevention of cancer in humans is still an open question.

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Diet, Epigenetics and Cancer Prevention

Emily Ho, PhD

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The classic view of cancer etiology is that genetic alterations damage DNA structure and induce mutations resulting in non-functional proteins that lead to disease progression. More recently, the role of epigenetic alterations during cancer has gained increasing attention. The reversible acetylation of histones is an important mechanism of gene regulation. During cancer progression, specific modifications in acetylation patterns on histones are apparent. Targeting the epigenome, including the use of histone deacetylase (HDAC) inhibitors, is a novel strategy for cancer chemoprevention. Recently, drugs classified as HDAC inhibitors, have shown promise in cancer clinical trials. Based on the similarity of sulforaphane (SFN) metabolites and other phytochemicals to known pharmacological HDAC inhibitors, we previously demonstrated that sulforaphane, a phytochemical derived from cruciferous vegetables, acts as an HDAC inhibitor in the prostate, causing enhanced histone acetylation, de-repression of P21 and Bax, and induction of cell cycle arrest/apoptosis, leading to cancer prevention. Other epigenetic mechanisms, including DNA methylation, histone methylation and non-coding RNA also appear to be impacted with SFN. This work suggests that phytochemical may have the ability to alter epigenetic events that lead to disease prevention. In human supplementation trials, we have directly compared the effects of the “whole food” (broccoli sprouts) to commercially available supplements. We have found a significant decrease in bioavailability and impact on HDACs with supplements compared to the whole food. Surprisingly, even when supplements are pre-treated with myrosinase, the release of sulforaphane from its glucosinolate precursors in the supplement is limited. These studies are significant because of the potential to qualify or change recommendations for high-risk prostate cancer patients and thereby increase their survival through simple dietary choices incorporating easily accessible foods into their diets. These mechanistic pre-clinical studies have provided a strong scientific foundation for on-going human clinical trials.
Can an Epigenetics Diet Prevent Cancer?

Trygve O. Tollefsbol, PhD, DO

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Nutrition Obesity Research Center
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Lead Editor and Founder, Elsevier’s Translational Epigenetics Series
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Interest in cancer epigenetics has arisen from the fact that epigenetic processes affect many aspects of tumor formation and progression. The reversibility of epigenetic changes that may arise in early tumorigenesis is an important aspect of their potential in approaches for cancer prevention. We coined the term “epigenetics diet” only 6 years ago based on numerous studies delineating the impact of bioactive dietary compounds on changes in the epigenome. There is aberrant gene expression due to epigenetic changes in all cancer types, so an approach to cancer therapy would be to use these bioactive dietary compounds as a means of not only neutralizing epigenomic aberrations as cancer treatment, but also cancer prevention. Although many of these phytochemicals are efficacious alone or in combination, some are only efficacious at considerably high doses not achievable by diet alone. Combinatorial studies are important to enhance our understanding of the interactions between various epigenomic-modifying dietary compounds. Compounds that display anticancer properties by themselves may act in an additive, synergistic, or even antagonistic manner in combination. The epigenetic mechanisms for these interactions are not fully understood, and are an area of increasing interest for the studies of the epigenetics diet. For instance, have found that both (-)-epigallocatechin-3-gallate (EGCG) from green tea and sulforaphane (SFN) from cruciferous vegetables are able to down-regulate telomerase in breast cancer cells. This occurs through epigenetic modifications of the promoter region of hTERT, the gene that encodes the catalytic subunit of telomerase. Since telomerase promotes tumor formation and is active in about 90% of cancers, this epigenetic inhibition of its catalytic subunit gene may have considerable potential in breast cancer prevention. Our studies also indicate that these bioactive dietary compounds are able to convert estrogen-receptor (ER)-negative breast cancer cells to ER-positive breast cancer cells treatable with tamoxifen or to prevent the formation of highly lethal ER-negative breast cancer. This occurs through epigenetic modifications of the ERα gene in response to EGCG and SFN. The components of the epigenetic diet are effective both in vitro and in vivo and also appear to be more effective when administered early in life. On the horizon for studies on the epigenetics diet and cancer prevention are additional combinatorial studies to prevent cancer, studies on the effects of the epigenetics diet on the gut microbiome and cancer prevention and early-life analyses of the impact of the epigenetics diet on cancer prevention as well as many other areas of study.
Session 6: Metabolism of Bioactives (Polyphenols)

Chair: Neil Shay, PhD, Food Science and Technology, Oregon State University, Corvallis, OR

Thursday, September 14th
10:00 AM - 12:00 PM
The metabolome of \([2-^{14}C]}(-)\)-Epicatechin in Humans: Implications for the Assessment of Efficacy, Safety, and Mechanisms of Action of Polyphenolic Bioactives

Hagen Schroeter, PhD

*Global Director of Fundamental Biomedical Research and the Flavanol Research Program, MARS, Inc.*

*Adjunct Research Professor, Department of Nutrition*

*University of California Davis, Davis, CA*

Necessitated by longer life expectancies globally and by rising health care costs, there is increasing interest in better understanding the impact of diet on health and healthy aging. However, comprehensive understanding of how specific food constituents affect human health is limited to a comparatively small number of essential nutrients. Relatively little is known about the impact on health of a large variety of non-essential dietary constituents (bioactives), which have been proposed to have significant effects on disease risk reduction and primary disease prevention. A critical assessment of available knowledge in this area highlights significant gaps and cross-disciplinary controversies. Examples of such gaps include the urgent need for biomarkers, i.e. objective measures of dietary intake; the need for assessing safety and risks associated with intake of bioactives; and the need for understanding the mechanisms of action of these compounds. Thus, to further advance our knowledge, we submit that we need to gain greater understanding of the absorption, distribution, metabolism and excretion (ADME) of bioactives. We selected here the flavanol \((-)\)-epicatechin (EC) as an example of a widely studied bioactive food constituent and investigated the ADME of \([2-^{14}C]}(-)\)-epicatechin (300 μCi, 60 mg) in humans (n = 8). We demonstrated that 82±5% of ingested EC was absorbed. We also established pharmacokinetic profiles and identified and quantified >20 different metabolites. The gut microbiome proved to be a key driver of the ADME of EC. Furthermore, we noted species-dependent differences in the metabolism of EC, an insight with perhaps significant consequences for investigating the mechanisms of action underlying the beneficial effects of EC. These differences will also need to be considered when assessing the safety of EC intake in humans. In addition, we identified a potential biomarker for the objective assessment of EC intake that could help to strengthen epidemiological investigations.
Cranberries are rich in potentially bioactive (poly)phenols, including proanthocyanins, anthocyanins, flavonols and phenolic acids. A limited number of studies have investigated the absorption, distribution, metabolism and excretion (ADME) of cranberry (poly)phenols in humans. Due to the difficulty and cost of obtaining authentic standards from commercial sources, the existing studies which investigated cranberry-derived phenolic acid metabolites have used either enzymatic treatment with glucuronidase and sulfatase to cleave the glucuronide and sulfate moieties from phase II metabolites, or have used the aglycone counterpart for quantification, which leads to inaccurate results. We have recently investigated the ADME of cranberry (poly)phenols using authentic standards. We found that cranberry (poly)phenols are more bioavailable than previously thought, with a large number of small phenolic and ring fission metabolites present in circulation in micromolar concentrations for at least 24 h post-consumption. Sixty metabolites were identified in plasma and urine, including cinnamic acids, dihydrocinnamic, flavonols, benzoic acids, phenylacetic acids, benzaldehydes, valerolactones, hippuric acids, catechols, and pyrogallols. Most of these metabolites are likely derived from gut microbial metabolism of proanthocyanidins and flavonoids, and to a lower extent from direct absorption of phenolic acids and breakdown of anthocyanins. Inter-individual variability of the plasma and urinary metabolite concentration was broad and dependent on the metabolite. Several of these metabolites were found to correlate with improvements in vascular function in a randomized controlled trial conducted in healthy individuals, including cinnamic acid derivatives, a flavonol glucuronide and a valerolactone sulfate. Our results indicate that cranberry (poly)phenols are bioavailable and gut microbial metabolism likely plays an important role in explaining their health benefits.
Use of Nutrigenomics to Decipher Mechanisms of Action of Polyphenols Mediating their Cardiovascular Protective Effect

Dragan Milenkovic, PhD

*Human Nutrition Unit*
*National Institute for Agricultural Research (INRA)*
*Clermont-Ferrand, France*

Epidemiological, human and animal studies suggest a protective role of dietary polyphenols against cardiovascular diseases. Their capacity to modulate genes expression and signaling pathways may be involved in their cardiovascular protective effects but the mechanisms remain still unknown. The aim of our research is to investigate the cardiovascular protective property of polyphenols and decipher underlying molecular mechanisms using holistic nutrigenomics approach. In murine models of atherosclerosis, 16-week supplementation with nutritionally-relevant doses of polyphenols (catechin, bilberry anthocyanins, naringin, or curcumin) decreased progression of atherosclerosis. Nutrigenomic studies of aorta indicated that polyphenols modified the expression of hundreds genes and functional analysis of these data identified a cluster of common pathways related to cell-cell adhesion, cell junctions, focal adhesion, and cell cytoskeleton. These processes regulate adhesion and transendothelial migration of monocytes into the intima of blood vessels, the initial step of atherosclerosis development. Immunofluorescence analysis of the aortic roots showed a reduction of the number of macrophages in intima.

To deepen molecular mechanisms of polyphenols, we investigated the impact of plasma metabolites of polyphenols on cell signaling pathways and miRNA expression in endothelial cells. The nutrigenomic effect in endothelial cells was associated with modulation of the phosphorylation level of several transcription factors and signaling pathway proteins, such as p38, p65 or Akt. We also observed changes in expression of miRNA in endothelial cells and bioinformatic analysis suggests that miRNA-target genes are also involved in processes of adhesion, transendothelial migration, focal adhesion or cytoskeleton organization. More recent studies allowed us to show that polyphenols can also modulate DNA methylation in endothelial cells, genes that are overrepresented in the pathways also regulating adhesion and transendothelial migration. Using different in-vitro assays, we have shown that these nutri(epi)genomic effects are associated with lowered monocyte adhesion to endothelial cells as well as transendothelial migration when exposed to polyphenol metabolites at physiologically-relevant concentrations. The role and impact of dietary polyphenols on endothelial function and gene expression in humans was investigated. We showed that orange juice decreases diastolic blood pressure and significantly improves postprandial micro-vascular endothelial reactivity and that hesperidin could be causally linked to the observed beneficial effect of orange juice. Nutrigenomics study revealed that orange juice and hesperidin consumption commonly modulated expression of 1,582 genes. Many of these genes were involved in chemotaxis, adhesion, infiltration and lipid transport, which is suggestive of a lower recruitment and infiltration of circulating cells to vascular wall and lower lipid accumulation.

In conclusion, these results provide evidence of new and complex mechanisms of action of polyphenols underlying their cardiovascular health properties and importance of use of system biology approach in this area of research.
Athero-protective Actions of Flavonoids

Roland Stocker, PhD

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Population studies suggest cardiovascular health benefits of consuming fruits and vegetables rich in polyphenolic compounds such as flavonoids. The association between dietary polyphenols and atherosclerosis has been confirmed to some extent by intervention studies with certain polyphenols or food extracts rich in polyphenols, which have shown improvements in cardiovascular risk factors such as vascular function and blood pressure. The mechanisms involved in the bioactivity of dietary polyphenols, or their metabolites, may include in vivo antioxidant activity (free radical scavenging), increase in nitric oxide bioavailability, and/or the induction of protective enzymes. We reported previously that the flavonoid quercetin protects arteries from oxidant-induced endothelial dysfunction and attenuates atherosclerosis in apolipoprotein E gene knockout mice, with induction of heme oxygenase-1 (Hmox1) playing a critical role. The present study investigated the structural requirements of flavonoids to induce Hmox1 in human aortic endothelial cells (HAEC). We identified ortho-dihydroxy groups and an α, β-unsaturated system attached to a catechol as the key structural requirements for Hmox1 induction. Active but not inactive flavonoids had a low oxidation potential and prevented ascorbate autoxidation, suggesting that Hmox1 inducers readily undergo oxidation and that oxidized, rather than reduced, flavonoids may be the biological inducer of Hmox1. To test this hypothesis, we synthesized stable derivatives of caffeic acid (3-(3,4-dihydroxyphenyl)-2-propenoic acid) containing either ortho-dihydroxy or ortho-dioxo groups. Compared with the dihydroxy compound, the oxidized (quinone) analog induced Hmox1 more potently in HAEC and also provided enhanced protection to arteries of wild type animals against oxidant-induced endothelial dysfunction. In contrast, the quinone analog failed to provide protection against oxidant-induced endothelial dysfunction in arteries of Hmox1−/− mice, establishing a key role for Hmox1 in vascular protection. These results suggest that oxidized forms of dietary polyphenols are the likely inducers of Hmox1 and may explain in part the protective cardiovascular effects of diets rich in these compounds.
Session 7: Nutrition and Brain Health

Chair: Gene Bowman, ND, MPH, Nestlé Institute of Health Sciences, Lausanne, Switzerland

Thursday, September 14th
1:30 - 3:30 PM
Alzheimer’s Disease (AD), the most common cause of dementia, currently affects 5.3 million adults in the USA. Recent increases in federal funding for AD research recognize the urgent need for better treatments for this disease. Current FDA approved drugs for AD are either cholinesterase inhibitors or glutamate receptor antagonists. However, these drugs do not influence disease progression. A signature pathological feature of AD is the presence of β-amyloid plaques in the brain, but in recent trials, anti-amyloid immunological agents have failed to show clinical cognitive benefits. The toxic sequelae of β-amyloid deposition have now been proposed as more relevant targets to limit AD progression. The targets include other known features of AD such as hyperphosphorylated tau protein, mitochondrial dysfunction, oxidative stress, and loss of synapses in affected brain regions. Centella asiatica (CA) is an edible plant that is consumed as a leafy green or component of juices in Asian countries. It also enjoys a strong reputation as a herbal nerve tonic and memory enhancer. Using multiple in vivo and vitro approaches, we have found that Centella asiatica (CA), a traditional herbal memory enhancer, not only improves cognition, but can improve mitochondrial activity, antioxidant response and dendritic arborization, and decrease tau phosphorylation. Active compounds in CA include triterpenes and caffeoylquinic acids. These studies will be described both to illustrate models applicable to the study of anti-AD botanicals, and to showcase CA’s potential as a disease-modifying, botanical agent for AD.
Effects of Diet on Gut-Brain Interactions

Kathy Magnusson, DVM, PhD

Professor, Department of Biomedical Sciences
College of Veterinary Medicine
Principal Investigator, Linus Pauling Institute
Oregon State University, Corvallis, OR

The body is covered by bacteria, including the lining of the digestive tract. The number of bacteria in the gut outnumbers the body’s own cells. It has been known for a long time that the bacteria play a role in digestion, but a new field of study focused on the gut microbiota is finding that these bacteria can influence many organ systems in the body, including diseases of the digestive tract, the immune system and even the brain. There is increasing evidence for two-way communication between the gut bacteria and the nervous system. The microbiota signal the brain in multiple ways, including via cytokines released by inflammation, release of neurotransmitters or precursors by bacteria or enteroendocrine cells, or via sensory neurons in the vagus nerve. The nervous system can influence bacteria via norepinephrine. Diet can have major impacts on the microbiota and behavior. High energy diets produce alterations in the microbiota that are associated with deficits in long-term memory and cognitive flexibility. Prebiotics, foods that can promote the abundance of certain types of bacteria, have effects on decreasing anxiety and depression. Microbial metabolites, such as propionate and 4-ethylphenylsulfate, are associated with autism spectrum disorders. These studies suggest that the interrelationship between diet and the gut microbiota can have important impacts on brain health.
Oxidized Lipids in Brain Trauma

Hülya Bayır, MD

Department of Critical Care Medicine, Safar Center for Resuscitation Research Department of Environmental and Occupational Health, Center for Free Radical and Antioxidant Health, University of Pittsburgh Children's Hospital of Pittsburgh of UPMC, Pittsburgh, PA

Traumatic brain injury (TBI) is a worldwide problem with high incidence in children and young adults. Fifty percent of surviving children with severe TBI have poor neurological outcome at six months. TBI sets into motion a cascade of biochemical and cellular events with activation of multiple pathways of neuronal death representing viable therapeutic targets. We discovered that selective peroxidation of a mitochondria-specific phospholipid, cardiolipin (CL), occurs in severe pediatric TBI and represents a required mitochondrial stage of neuronal apoptosis. We further identified cytochrome c (cyt c) as a catalyst of CL peroxidation occurring via the formation of cyt c/CL complexes with peroxidase activity triggered by H$_2$O$_2$. Thus cyt c/CL redox interactions and CL peroxidation represent a missing causal link between known reactive oxygen species production and mitochondrial pro-apoptotic responses. Corroborating with this, a mitochondria-targeted small molecule inhibitor of CL peroxidation suppressed TBI-induced apoptosis in vivo and preserved cognitive function in postnatal day (PND) 17 rats. In conclusion the ability to selectively modulate CL oxidation, a critical early event in the mechanism of apoptosis, could lead to targeted therapies for TBI and ultimately improve outcome for children after brain injury.

Support: NIH (NS061817, NS076511, NS084604, AI068021)
Alzheimer’s disease is considered a disease of accelerated aging, and it is likely that pathogenic processes begin decades prior to changes in brain atrophy and cognitive decline are detected in clinic. Seizures are an important shared pathological feature of several neurodegenerative diseases that can dramatically increase cognitive decline by triggering apoptosis and cell death in hippocampus and cortical regions critical for learning and memory. Damage can occur even in the absence of large or observable seizures. Ascorbic acid (ascorbate, vitamin C) deficiency may contribute directly to the neurodegenerative changes that accompany seizures via the glutamate uptake-ascorbic acid release exchange mechanism in astrocytes. Low brain ascorbate altered expression of several genes involved in glutamate transport in the hippocampus, most notably GLT-1. Ascorbate deficient mice and mice carrying familial Alzheimer’s disease mutations (APP\textsubscript{SWE}/PSEN1\textsuperscript{DE9}) are more sensitive than wild-type controls to kainic acid-induced seizures, even at very low doses. Accelerated and prolonged response patterns were observed in both EEG and behavioral studies. Upregulation of GLT-1, protected against acute kainic acid-induced immobility, but only in mice with adequate ascorbate levels. Even a single, very mild seizure negatively impacted learning and memory in the water-maze in APP\textsubscript{SWE}/PSEN1\textsuperscript{DE9} mice compared to wild-type controls. Together these studies support the role of ascorbate deficiency in increased seizure susceptibility, which may be particularly relevant in Alzheimer’s disease.
Session 8: Lightning Talks

Chair: Viviana Pérez, PhD, Linus Pauling Institute, Oregon State University

Thursday, September 14\textsuperscript{th}
4:00 - 5:00 PM

Abstracts for the Lightning Talks can be found in the Poster Sessions
Session 9: Mechanisms of Vitamin C in Cancer

Chair: Anitra Carr, PhD, University of Otago, Christchurch, New Zealand

Friday, September 15th
8:00 - 10:00 AM
Recommended intakes for many vitamins are based on preventing deficiency, with a safety margin. Our hypotheses are that vitamin recommendations can and should be based on detailed, state-of-the-art physiology investigations in humans, using clinical tools of concentration-function relationships and pharmacokinetics, coupled to cell biology and genomics. Clinical investigation tools were used to characterize vitamin C physiology in healthy men and women, ages 18-28 years. Utilizing a depletion-repletion design, vitamin C concentrations were found to be tightly controlled in plasma and cells, over a dose range of 30 to 2500 mg. Tight control had at least 4 components: bioavailability, or intestinal absorption; tissue transport; renal filtration, or renal reabsorption/excretion; and utilization. The first three components were regulated by two tissue transporters, mediated by identified transporters SLC23A1 (SVCT1) and SLC23A2 (SVCT2), as well as by as yet unidentified transporters. Recent studies indicate that red blood cells are a unique tissue compartment for ascorbate. In contrast to other tissues, red blood cells transport dehydroascorbic acid (oxidized ascorbate), utilizing GLUT1. Bioavailability studies showed that percent intestinal absorption decreased as doses increased. Intravenous administration of doses above 100 mg produced ascorbate plasma concentrations that could not be achieved with oral dosing. These data indicated that, depending on dose and rate of administration, intravenous ascorbate produced pharmacologic plasma concentrations, with renal filtration restoring homeostasis. Pharmacologic intravenously administered ascorbate but not oral ascorbate had potential to decrease cancer growth in humans. Pharmacologic ascorbate was cytotoxic to cancer but not normal cells in vitro, in animals, and in small but encouraging studies in humans. Pharmacologic ascorbate mediated cancer cell death by generation of extracellular hydrogen peroxide (H₂O₂) in vivo. Pharmacologic ascorbate can be considered a pro-drug for delivery of pharmacologic H₂O₂ concentrations to the extracellular space. There are an ever-increasing multiplicity of downstream mechanisms of ascorbate-mediated cancer cell cytotoxicity that are H₂O₂-dependent. In specifically modified cell lines, mechanisms have been proposed that are H₂O₂-independent, for example based on dehydroascorbic acid. However, this mechanism does not appear to have general applicability. Clinically, pharmacologic ascorbate has a surprisingly strong safety profile. Non-specificity, or promiscuity, of many oncology therapeutics is often harmful because of collateral damage to normal tissues in humans. In contrast, benefit is provided to patients by the promiscuity of pharmacologic ascorbate because of its safety and potential efficacy. Accelerated ascorbate utilization occurs in critically ill patients. Using similar pharmacokinetics principles as for cancer treatment, intravenous ascorbate has shown recent promise in treatment of sepsis. Based on transporter principles and RBC physiology, ascorbate either orally or intravenously has additional promise in delaying complications of diabetic microvascular disease. Considered together, the data indicate that exhaustive characterization of vitamin physiology in healthy people serves as a gateway to advances in disease treatment and prevention. Applying this approach to other vitamins will provide physiologic bases for vitamin recommendations, and may reveal unanticipated application to disease treatment.
Vitamin C-dependent Regulation of the Hypoxic Response in Cancer

Margreet C.M. Vissers, PhD

Centre for Free Radical Research
Pathology Department
University of Otago, Christchurch, New Zealand

Rapid tumor growth initiates hypoxic stress and activates the transcription factor hypoxia-inducible factor (HIF)-1, promoting angiogenesis, glycolysis and enhanced resistance to radio- and chemotherapy. HIF-1 is down-regulated by oxygen-sensing hydroxylases that require vitamin C (ascorbate) as cofactor and we have observed an inverse correlation between cell ascorbate levels and HIF-1 activity. Our hypothesis is that poor vascular ascorbate delivery in a growing tumor limits cell ascorbate content and augments the hypoxic response, thereby driving tumor growth. Raising tumor ascorbate could reverse this effect. Our in vitro modelling of ascorbate uptake into tissues is consistent with this hypothesis and suggests that supra-physiological concentrations are required to saturate tumor tissue.

We have shown that HIF-1 is moderated by intracellular ascorbate in tumors grown in the Gulo−/− mouse, a model of human vitamin C dependency. Pre-clinical studies with tumor tissue from cancer patients have also shown a correlation between cellular ascorbate levels, HIF-1 activity, tumor size and patient outcome in breast, colorectal, endometrial and renal cancers. These results suggest that raising tumor ascorbate could slow tumor growth by moderating HIF-1 activation. Our data from tumor-bearing Gulo−/− mice suggests that achieving mM plasma ascorbate levels by daily administration of supra-physiological doses exerts an anti-tumor effect, with decreased HIF-1 and vascular endothelial growth factor protein expression as well as reduced microvessel density, tumor hypoxia and tumor growth.

We have recently completed a pilot study with colorectal cancer patients who were given high dose vitamin C (1g/kg) for four days prior to surgical removal of the tumor. Our analysis of the tumor tissue indicates that a significant increase in ascorbate levels is achieved through the high dose intervention that could impact on HIF-1 activation. Together, our results support the suppression of HIF-1 as an anti-tumor activity of ascorbate that may be useful in a clinical setting.
High dose intravenous ascorbate (IVC) has attracted increasing interests as a low-toxic cancer therapy. IVC bypasses bioavailability barriers of oral ingestion, provides pharmacologic concentrations in tissues, and exhibits selective cytotoxic effects in cancer cells through peroxide formation. The selectivity is related to the mechanisms of action. We postulate that ascorbate-induced ROS have multiple mechanisms of action that preferably influence cancer cells. First, ascorbate-generated ROS induces DNA damage. Downstream to DNA damage, cellular NAD+ decreases as an effect of PARP activation. Decrease of NAD+ inhibited GAPDH activity and depletes ATP in cancer cells, while normal cells maintain their ATP levels. This phenomenon has a root in dysregulated glucose metabolism in cancer cells, known as the Warburg Effect, that cancer cells depend on a larger proportion on glycolysis for ATP whereas normal cells depend more on oxidative phosphorylation. Second, lack of NAD+ inhibits activity of Sirt-2, a tubulin deacetylase, and therefore increases tubulin acetylation, which in turn disrupts dynamics of microtubules. This influences cancer cells that are actively undergoing mitosis and migration. Third, when PARP is inhibited, excessive DNA damage results in cell death. Further, ascorbate inhibited EMT, an important process contributing to cancer metastasis. Finally, ascorbate enhanced collagen synthesis in tumor stroma. Despite the controversial reports on the effect of elevated collagen in tumor progression, the increased collagen by ascorbate treatment is associated with restriction of tumor invasion in our animal experiment and in patient.

Taken together, these data show multi-targeting effects of ascorbate that favor death/inhibition in cancer cells relative to normal cells. With minimal toxicity, the multi-targeting mechanism of ascorbate is advantageous because it could decrease the likelihood of resistance, and provides multiple opportunities for combining with standard chemo and radiation therapies.
Bone marrow stem cell transplantation is used in the treatment of patients with cancer, mostly hematological types of cancers. Both autologous transplants as well as allogeneic transplants can be used. Autologous transplantation is used to recover bone marrow after high dose chemotherapy with or without radiotherapy. Allogeneic transplantation is used to induce a graft versus tumor response against the patients’ malignant cells, based on an immunological mismatch between donor and recipient. Both treatments do induce a time period of immunosuppression in which the patient is at risk for infections. In autologous transplants, this risk is relatively low (mortality 2-5%). In allogeneic transplants, this risk is higher and partly depending on the source of the transplant that might lead to a state of immune-deficiency off over 3 months.

This clinical question led us to study the possible option of add back of T and/or Natural Killer (NK) cells cultured from stem cells in vitro. In vitro it is possible to culture (pre-) T cells from stem cells in the presence of feeder cells transfected with Notch-Ligands, that are crucial for T cell development. In order to test a feeder free system for GMP accredited clinical use, we observed that different media gave different results: the difference to be explained by the presence or absence of vitamin C. In the presence of Vitamin C double-positive T cells from stem cells can be obtained in the absence of stromal cells. However, for the further development to single positive T cells, stromal cells are still needed. In addition, we observed that also NK cells derived and matured faster from stem cells as well as from early pre T/NK cells in the presence of Vitamin C. These cells are completely functional NK cells.

Based on these in vitro data we went back to the clinic to see if patients that are treated for hematological diseases, with or without a stem cell transplantation, might have low vitamin C levels. Patients had lower Vitamin C levels (20.4 µM; N=42) compared to 65 µM in controls. 20% of the patients had levels below 11 µM or undetectable, considered to be Vitamin C deficient. In a more recent prospective study we do confirm this observation: patients do have lower vitamin C levels while on treatment compared to family members as controls.

These data are the basis for an intervention study using Vitamin C to see if leukocyte recovery and therefore infections, morbidity and mortality can be improved after stem-cell transplantation for hematological malignancies.
Session 11: Vitamin C Therapy in Cancer

Chair: Ramesh Natarajan, PhD, Virginia Commonwealth University, Richmond, VA

Friday, September 15th
10:30 AM - 12:30 PM
Ascorbate functions as a versatile reducing agent. At pharmacological doses (P-AscH-, [plasma] =20 mM), achievable through intravenous delivery, oxidation of AscH- can produce a high flux of H2O2 in tumors. Normal cells/tissues seem not to be affected by an increased flux of H2O2, while exposure to an increased flux of H2O2 is detrimental to many cancer cells. I will address three basic issues for the use of P-AscH- in the treatment of cancer: (1) the oxidation of ascorbate to produce a flux of H2O2; (2) catalase as the first-line defense of cells against an increased flux of H2O2; and (3) potential downstream modulators of the cellular response to this oxidative challenge. Our laboratory is quantitatively addressing these three aspects of the potential use of P-AscH- to treat cancer. I will show how data from experiments that address these issues are used to guide clinical trials.

The encouraging data from our basic science efforts have underpinned six clinical trials on the use of P-AscH- as an adjuvant to the standard of care at The University of Iowa; 1 completed (Phase 1, pancreatic cancer with gemcitabine); 1 terminated (Phase 2, pancreatic cancer with gemcitabine); 2 active, but recruiting is finished (Phase 1, pancreatic cancer with gemcitabine and radiation; Phase 1, glioblastoma multiforme, temozolomide and radiation); 2 active and recruiting (Phase 2, Non-Small-Cell Lung Cancer Paclitaxel, Carboplatin); Phase 2, glioblastoma multiforme, temozolomide and radiation). An overview of current results will be presented.

Conclusions: From our basic science results, P-AscH- may be and effective adjuvant for some standard of care therapies; P-AscH- is safe as an adjuvant with the chemotherapeutic agents we have tested as well as with radiation; adverse events are minimal and mild; there are suggestions of efficacy.
Epigenetic Treatment of Cancer by Vitamin C

Gaofeng Wang, PhD

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Recent advances have uncovered a previously unknown function of vitamin C in regulating the demethylation of DNA and histones. Ten-eleven translocation (TET) dioxygenases initiate DNA demethylation by converting 5-methylcytosine (5mC) into 5-hydroxymethylcytosine (5hmC). Vitamin C is essential for the function of TET by providing Fe(II), a cofactor of TET. Loss of 5hmC is accompanied with malignant cellular transformation. Overexpressing TET can partially re-establish a normal 5hmC profile in cancer cells and represses their malignancy. While overexpressing TETs in patients might not be clinically feasible, these discoveries suggest that finding a means of restoring normal 5hmC content may yield a novel therapy for cancer. The expression of vitamin C transporter SVCT2 is frequently downregulated in cancer. For instance, SVCT2 expression is decreased in 72.5% of breast cancer cases by at least 1.5-fold compared to the matched normal breast tissues. This suggest it is necessary to compensate the downregulated SVCT2 with vitamin C supplements in risk population and cancer patients. Treatment of cancer cells with vitamin C increases 5hmC content and results in a markedly shifted transcriptome. These changes are correlated with decreased cellular malignant phenotypes. Furthermore, by promoting the demethylation of DNA and histones, vitamin C changes the response of cancer treatment. For example, vitamin C improves the efficacy of Bromodomain and extraterminal domain inhibitors (BETi) in treating melanoma. In conclusion, vitamin C can prevent cancer initiation and progression by reestablishing 5hmC. Vitamin C also can improve the response of certain cancer drugs.
Intravenous Vitamin C and Chemotherapy in Ovarian Cancer

Jeanne A. Drisko, MD

Director, KU Integrative Medicine
Riordan Endowed Professor of Orthomolecular Medicine,
University of Kansas Medical Center, Kansas City, KS

Background: Ascorbate (vitamin C) has long been used as an unorthodox therapy for cancer, even though the underlying scientific mechanisms are not well understood.

Clinical Trial: A pilot phase 1/2a clinical trial was conducted in patients with newly diagnosed stage III or IV ovarian cancer. High-dose intravenous ascorbate (AA) was added to conventional paclitaxel/carboplatin (Pax + Cp) therapy, and toxicity was assessed. Twenty-seven participants were randomized into either the standard Cp + Pax arm or the Cp + Pax + AA arm. Cp + Pax chemotherapy was administered for the initial 6 months, and AA treatment for 12 months. Any and all unwanted events were counted and graded for severity according to NCI CTCAEv3. Records for adverse events include patient interviews, emergency room visits, patients’ oncologist visits, and hospitalization records. The number of adverse events in each grade for each participant was divided by the number of encounters of that participant, and then the adverse events per encounter were averaged in the Cp + Pax arm and the Cp + Pax + AA arm, respectively. Participants were followed for survival for 5 years.

Statistical Analysis: Two-tailed Student’s t-test was performed for toxicity comparison between chemotherapy group and chemotherapy + ascorbate group. Welch’s t-test was used when the variances in the two compared populations were unequal. A log-rank test was performed for comparison of the survival curves between the chemotherapy group and the chemotherapy + ascorbate group.

Results: Ascorbate worked synergistically in vitro and in vivo with the first-line chemotherapeutic drugs carboplatin and paclitaxel. In patients with advanced ovarian cancer, treatment with ascorbate reduced toxicities associated with chemotherapy. Because the study was not powered for detection of efficacy, statistical improvement in survival was not observed.

Conclusion: Given the advantage of low toxicity of ascorbate, larger clinical trials need to be done to definitively examine the benefit of adding ascorbate to conventional chemotherapy.
Challenges of Natural Product Drug Development

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Numerous drugs that the US Food and Drug Administration (FDA) has approved for use in cancer therapy are derived from plants, including taxanes such as paclitaxel and vinca alkaloids such as vinblastine. Dietary supplements are another category of natural products that are widely used by patients with cancer, but without the FDA-reviewed evidence of safety and efficacy—be it related to survival, palliation, symptom mitigation, and/or immune system enhancement—that is required for therapy approval. Nearly half of patients in the United States with cancer report that they started taking new dietary supplements after being given a diagnosis of cancer. Oncologists are challenged in providing advice to patients about which supplements are safe and effective to use to treat cancer or the side effects of cancer therapy, and which supplements are antagonistic to standard treatment with chemotherapy, radiation, and/or immunotherapy. Despite the large number of trials that have been launched, the FDA has not approved any dietary supplement or food to prevent cancer, halt its growth, or prevent its recurrence. We will review the primary challenges faced by researchers attempting to conduct rigorous trials of natural products, including shortages of funding due to lack of patentability, manufacturing difficulties, contamination, and lack of product consistency. We will also highlight the methods used by dietary supplement marketers to persuade patients that a supplement is effective (or at least safe) even without FDA approval, as well as the efforts of the US government to protect the health and safety of its citizens by ensuring that the information used to market natural products is accurate. Finally we will close with a summary of the most widely used databases of information about the safety, efficacy, and interactions of dietary supplements.
Session 12: Vitamin C Therapy in Sepsis

Chair: Margreet Vissers, PhD, University of Otago, Christchurch, New Zealand

Friday, September 15th
2:00 - 4:30 PM
Intravenous Vitamin C in Pre-Clinical Sepsis and Trauma Models

Ramesh Natarajan, PhD

Professor of Medicine, Department of Internal Medicine, Division of Pulmonary Disease and Critical Care Medicine, Virginia Commonwealth University, Richmond, VA

Bacterial infections of the lungs and abdomen are among the most common causes of sepsis. Sepsis-induced acute lung injury (ALI) is a persisting clinical problem with no direct therapy. We used various models of sepsis in wild type and knockout mice to determine whether parenteral vitamin C modulates the dysregulated pro-inflammatory, pro-coagulant state that leads to sepsis-induced lung injury. Male C57BL/6 wild type mice and mice lacking functional L-gulono-γ-lactone oxidase (Gulo−/−) were exposed to bacterial lipopolysaccharide (endotoxin) or a fecal stem solution (polymicrobial sepsis) to induce abdominal peritonitis 30 min prior to parenterally receiving either reduced vitamin C (ascorbic acid, 200 mg/kg) or oxidized vitamin C (dehydroascorbic acid, 200 mg/kg). Variables examined included survival, extent of ALI, pulmonary inflammatory markers, bronchoalveolar epithelial permeability, alveolar fluid clearance, epithelial ion channel, and pump expression, tight junction protein expression, cytoskeletal rearrangements, neutrophil extracellular trap formation (NETosis), multiple organ failure and various coagulation parameters in septic blood. Sepsis induced ALI was characterized by compromised lung epithelial permeability, reduced alveolar fluid clearance, pulmonary inflammation and neutrophil sequestration, increased formation of NETs, significant coagulation abnormalities, and increased mortality due to multiple organ failure. A single infusion of parenteral vitamin C protected mice from the deleterious consequences of sepsis by multiple mechanisms, including attenuation of the NFκB driven pro-inflammatory response, enhancement of epithelial barrier function, increasing alveolar fluid clearance, prevention of sepsis-associated coagulation abnormalities, attenuation of NETosis, and normalization of physiological functions that attenuated the development of multiple organ dysfunction. These pre-clinical studies using parenteral vitamin C were central to the completed PHASE I trial of intravenous vitamin C in sepsis and the ongoing PHASE II multi-center trial examining the efficacy of intravenous vitamin C infusion in human sepsis-associated ALI.

New data from a model of hemorrhagic shock and polytrauma show that IV vitamin C may mitigate the pro-inflammatory/pro-coagulant response that contributes to multiple organ failure following acute severe polytrauma.
**Intravenous Vitamin C as Therapy for Sepsis-Induced Acute Lung Injury**

Alpha A. (Berry) Fowler, III, MD

*William Taliaferro Thompson Professor of Medicine, Division of Pulmonary Disease and Critical Care Medicine, Department of Internal Medicine, Virginia Commonwealth University School of Medicine, Richmond, VA*

The incidence of sepsis and sepsis-associated organ failure continues to rise in American Intensive Care Units. Over 1 million cases of sepsis (i.e., bacterial, fungal, viral) occur in the U.S. population each year. Some 375,000 patients will die from sepsis either primarily from septic shock or secondarily from organ failure. Sepsis-induced organ failure contributes cumulatively to patient mortality. Patients with severe sepsis suffer higher mortality rates compared to patients with organ failure but no sepsis. Despite over 15,000 patients studied and over 1 billion dollars in study costs, effective sepsis therapy remains elusive. Clinical trials that have targeted mediators of inflammation or coagulation such as rosuvastatin or activated protein C have not reduced septic mortality, suggesting that single-target therapy fails to meet the challenges of complex multicellular activation and interactions. Recent studies suggest that ascorbic acid may attenuate pathological responses in septic microvasculature. In preclinical studies, ascorbic acid improved capillary blood flow, microvascular barrier function, and arteriolar responsiveness to vasoconstrictors in septic animals. We showed that parenterally administered ascorbic acid attenuated vascular lung injury in septic mice. Subnormal plasma ascorbic acid concentrations in septic patients correlates inversely with multiple organ failure and directly with survival. We report in this presentation that intravenous Vitamin C is safe to administer to patients with severe sepsis, that it improves sepsis-induced organ failure and that it attenuates biomarkers of systemic inflammation and vascular injury. We also report aspects of the ongoing NIH-sponsored trial: Vitamin C Infusion for Treatment In Sepsis Induced Acute Lung Injury (CITRIS-ALI).
Vitamin C Requirements and Mechanisms of Action in Severe Infection

Anitra Carr, PhD

Department of Pathology
University of Otago
Christchurch, New Zealand

Patients with severe infections, such as pneumonia, can develop sepsis, an uncontrolled inflammatory response to the initial infection. This can result in organ failure and septic shock, the major cause of death of critically ill patients in intensive care. We and others have found that critically ill patients have severely depleted vitamin C levels despite recommended intakes via liquid nutrition. One study has shown that critically ill patients require parenteral vitamin C at levels that are 30-fold higher than recommended intakes. Patients with sepsis have dysregulated immune function, including compromised leukocyte function. Neutrophils are the primary responders to infection and these cells are known to accumulate high levels of vitamin C, suggesting an important role for the vitamin in immune cell function. We and others have shown that vitamin C can enhance neutrophil chemotaxis, oxidant production, apoptosis and clearance by macrophages. Vitamin C is an important cofactor for numerous biosynthetic and regulatory enzymes in the body. Because vitamin C is a cofactor for the enzymes that synthesize noradrenaline and vasopressin, we hypothesized that vitamin C administration to patients with severe sepsis and septic shock may decrease the need for exogenous administration of these vasopressors. Support for the vitamin C and vasopressor hypothesis comes from recent clinical trials which showed decreased vasopressor requirements in patients who received intravenous vitamin C. We are currently implementing a clinical trial in Christchurch Hospital ICU to assess the outcomes and mechanisms of action of intravenous vitamin C in severe sepsis.
A large body of experimental data has demonstrated that both corticosteroids and intravenous vitamin C reduce activation of nuclear factor κB (NF-κB) attenuating the release of pro-inflammatory mediators, reduce the endothelial injury characteristic of sepsis thereby reducing endothelial permeability and improving microcirculatory flow, augment the release of endogenous catecholamine’s and enhance vasopressor responsiveness. In animal models these effects have resulted in reduced organ injury and increased survival. Corticosteroids have been evaluated in several clinical trials, with meta-analysis of these trials demonstrating somewhat conflicting outcomes. Low-dose stress corticosteroids have proven to be safe with no increased risk of clinically important complications. While corticosteroids decrease vasopressor dependency the effect on the risk of developing organ failure and survival is less clear. Similarly intravenous Vitamin C has been evaluated in unselected surgical ICU patients, patients with burns those with pancreatitis and in two pilot studies of patients with severe sepsis and septic shock. In general these studies have demonstrated a reduction in the risk of multisystem organ failure (MSOF) although the effect on mortality is less clear. However, IV vitamin C was shown to be extremely safe with no recorded complications.

In vitro data has suggested that vitamin C and hydrocortisone may act synergistically. Barabutis et al. have demonstrated that hydrocortisone together with vitamin C protects the vascular endothelium from damage by endotoxin while neither agent alone had this effect. [2] Based on these clinical and experimental data we initiated a treatment protocol for patients with severe sepsis and septic shock that included intravenous vitamin C, hydrocortisone and thiamine. We have demonstrated that this therapeutic cocktail reverses the organ dysfunction of sepsis with a marked reduction in mortality.[3]

References

Session 13: Public Session

Chair: Maret Traber, PhD, Linus Pauling Institute, Oregon State University

Saturday, September 16th
9:00 - 11:00 AM

Intravenous Vitamin C and Cancer Treatment: Does It Work?

Jeanne A. Drisko, MD

Director, KU Integrative Medicine
Riordan Endowed Professor of Orthomolecular Medicine, University of Kansas Medical Center, Kansas City, KS

Diet and Cancer Prevention

Emily Ho, PhD

Professor, Endowed Director of the Moore Family Center for Whole Grain Foods, Nutrition & Preventive Health
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Oregon State University, Corvallis, OR