

Oxygen Club of California -- OCC2010

□ Oxidants And Antioxidants In Biology: *Translational Redox Science*

co-sponsored by the **Linus Pauling Institute**

Wednesday, March 17 – Saturday, March 20, 2010 □

Santa Barbara, California

Unofficial Meeting Notes by John D. Furber

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Introduction: The 2010 meeting of the **Oxygen Club of California** was held

□ Wednesday, March 17 – Saturday, March 20, 2010 at the Fess Parker Resort in Santa Barbara, California. The OCC was founded in 1994. It has been meeting in alternate years in Santa Barbara and at the **Linus Pauling Institute** in Oregon.

Disclaimer: These are my informal notes from the meeting. They are definitely incomplete, and probably contain some errors. I welcome comments and feedback, especially if you spot errors. Permission is granted to the Oxygen Club of California to use or adapt these notes for their newsletters and web site.

The complete program, abstracts of the talks, and many photographs from the meeting are available for download on the OCC website <http://www.oxyclubcalifornia.org>

More information on the work of any presenter can be found by searching PubMed or Google Scholar or Scirus, and reading their papers. Consequently, my notes here are not a comprehensive chronicle, but rather a sketchy introduction to people and ideas that you might wish to investigate further.

- There will be a meeting on *Nutrition, Oxygen Biology and Medicine* in Paris, 15-17 June 2011. <http://www.oxyclubcalifornia.org/Paris2011/nutrition-paris11.php>
- The next *Diet and Optimum Health* conference will be in Corvallis Oregon, in September 2011.
- There will be an *OCC meeting* in June 2012 in Alba, Italy. Watch the OCC website for details to be announced.



Overview and Highlights

Session 6 of this meeting was specifically on aging. But in addition, the other 5 sessions covered topics pertinent to aging, signaling, nutrition, and health.

In order to present a large number talks without parallel sessions, each talk was limited to 30 minutes, including time for questions. This works well, and keeps things moving. I commend the organizers for allowing participants to see all of the talks without parallel sessions.

Maret Traber took office as the incoming President of the OCC.

Susanne M. Henning, Chemoprevention by pomegranate and green tea: Drinking one cup of pomegranate juice per day slows the increase in PSA in men. There are beneficial active



ingredients in the skin and the connective fibers between the seeds of the pomegranate, so eat the skin and white pulp. The commercial juices and extracts squeeze the whole fruit, so you do get these benefits. Ellagitannins (ET) are in pomegranate, walnut, persimmon, strawberry, and other berries. Drinking brewed green tea inhibits xenograft tumor growth in mice.

Ana Maria Cuervo: During starvation, the cell obtains proteins by autophagy. But autophagy is also a mechanism of quality control. If the autophagy function fails, then the cell can die. Degradation of lipids by autophagy (Susmita Kaushik) can provide chemical energy to the cell. They call it "Macrolipophagy". Triglycerides => fatty acids => β -oxidation => energy. So blockage of macroautophagy (by blocking Atg7) results in an increase in lipid droplets in the cell. (Singh Nature 2009) (Koga FASEB 2010).

We see altered macroautophagy (MA) in Alzheimer's Disease. Autophagic vesicles (AV) accumulate in affected neurons. We see a defect in the pH of the lysosomes, so autophagosomes containing mitochondria fuse with lysosomes, but they do not degrade. (Lee, Cuervo, Nixon, et al Cell 141, 1146-1158, June 25, 2010)

We must identify compounds that can reactivate autophagy and resolve the problem.

[JDF: In older, nondividing cells, such as neurons and heart muscle, lysosomal digestion appears to be inhibited by accumulation of lipofuscin in lysosomes. Getting rid of that accumulation is an important unsolved problem, which when solved, would enhance lysosomal digestion, and I expect also enhance the effectiveness of autophagy.]

Tilman Grune: If unfolded proteins stay very long in the cell, they form initial aggregates. After time, they crosslink and form lipofuscin. This stuff can fill up to 70% of the volume of some (nondividing) neurons in elderly people.

How are intracellular AGEs degraded? The proteasome can not



degrade any of them. Oxidized proteins are easier for the proteasome to degrade, but not AGE-proteins. Cathepsin D and Cathepsin B are lysosomal digestive enzymes, which are best at degrading AGE-modified proteins, such as methyl glyoxal.

Chronic oxidation stress leads to nuclear protein oxidation and Stress Induced Premature Senescence (SIPS).

Harald zur Hausen: Novel Infectious Agents in Human Carcinogenesis: State and Perspectives

Many cancers are caused by various viruses and parasites. 21% of global cancer incidence is caused by infections: 0.8% of that by parasites; 35% of that by bacteria; 65% of that by viruses.

José (Pepe) Viña, Department of Physiology, University of Valencia, Valencia, Spain.

Exhaustion is not good for you. Exhaustion causes oxidative stress.

Bruce N. Ames: We require about 40 micronutrients, including amino acids, vitamins, 15 minerals, and 2 essential fatty acids (EFAs). A moderate deficiency ages you somewhat. Mg is found in chlorophyll. 56% of Americans are low in Mg. (Killilea PNAS 105:5768-5773, 2008) The human body wants to keep a ratio of 2Ca/1Mg. Mg deficiency causes DNA cross links. Even though half of Americans take multivitamins, they do not have enough Ca or fish oil because the pill would be too big. Depletion of any of the micronutrients causes DNA damage. Too much iron or too little iron is harmful. Men eating too much red meat get too much iron. Many women and children do not get enough iron.

(Ames. Triage Theory. PNAS 103:17589-594 Nov 2006)

People who eat natto (a fermented soybean food from Japan) have almost no heart disease.

Bharat B. Aggarwal: NFκB is the major mediator of inflammation. Many plant products block NFκB, including diferuloylmethane (curcumin from turmeric), katuka, goldenseal, pinitol talisapatak, resveratrol, Indol-3-carbinol (from broccoli), tocotrienol (from coconut), (but not tocopherol), spices, onion, fennel, rosemary, red chili, ginger zerumbone, black cumin, fenugreek, and fennel.

Young-Joon Surh: Resveratrol suppresses growth of human ovarian cancer cells. Garlic diallyl sulfide protects against H. pylori bacteria, which can cause stomach ulcers and other problems.

Georg Lietz, Human Nutrition Research Centre, School of Agriculture, Food and Rural Development, Newcastle University, Newcastle Upon Tyne, UK. Georg.lietz@ncl.ac.uk

A new **Micronutrients Genomics Project** aims to build a bioinformatics portal. Contact Georg to get involved.

Lester Packer: There is now a portable laser scanner that can assess the carotenoid status of



people by scanning their skin.

Tomas A. Prolla, Resveratrol mimics caloric restriction and retards aging in the heart.

Age-related hearing loss is halted by CR. More Sirt1 is not necessarily better. Increased Sirt1 expression is associated with prostate cancer cells. Exercise is as effective as CR at changing gene expression in cardiac tissue.

Geoffrey C. Gurtner, Department of Surgery, Stanford University School of Medicine, Calif

With aging, we see an impaired ischemic response in wound healing. Aging impairs new blood vessel growth, but not baseline vascular density. Aging impairs tissue ischemia-induced SDF-1 expression, and other wound healing genes. Aging impairs HIF-1 α stabilization directly and leads to necrosis of tissue. In diabetes and hyperglycemia, we see a similar event. Diabetes plus aging compounds the problem. Desferoxamine (DFO) chelates iron and salvages impairment of HIF 1 α , preventing necrosis, enhancing wound healing and neovascularization.

Dipak K. Das: Resveratrol at low concentration activates Atg5 and autophagy.

Tory M. Hagen: R- α -Lipoic Acid (R-LA) improves hepatic antioxidant capacity, which otherwise declines with age. It does this by reversing the age-related loss of endogenous antioxidants, especially GSH. Normally, the nuclear level of NF-E2-related-factor-2 (Nrf2), a transcription factor that regulates expression of GSH-synthesizing enzymes, markedly declines with age, but R-LA reverses this loss. Feeding old rats for 2 weeks 0.2% LA reverses the age-related loss of GSH. They resist toxic peroxides just like young rats.

Several speakers reported improved wound healing with hyperbaric oxygen treatment (HBO).

Omaida Velazquez, University of Miami, Florida, USA

Diabetic non-healing wounds are a major problem, leading to amputations and limb loss. Endothelial progenitor cells (EPC) participate in wound healing. They are mostly from bone marrow. EPCs can be extracted, expanded, and reinjected. They are already in phase 2 clinical trials. Hyperbaric oxygen (HBO) increases EPC mobilization. SDF-1 α increases EPC homing. Together, they increase wound healing in diabetic wounds.

Junji Yodoi, University of Kyoto, Japan.

Food with TRX-extract, derived from sake processing, appears to have some benefit in protecting the mucosal membranes of mice from smoke.

Hallway Conversations

Jennifer Ehren: Adenylate cyclase type 5 KO mice live 30% longer. They eat more and weigh less.

Fess Parker passed away on 18 March 2010 at the age of 85. Fess was a beloved actor, and the owner of the resort where the Oxygen Club of California usually meets.



OCC 2010 WORLD CONGRESS

Speakers and Notes

Wednesday, 17 March 2010, Keynote Lectures

Welcome: *Chandan K. Sen* and *Lester Packer*

New Horizons in HIV/AIDS

Luc Montagnier, World Foundation AIDS Research & Prevention, UNESCO, Paris, France

Notes: Oxidative stress weakens the immune system. There is evidence of oxidative stress in HIV infections and AIDS. Oxidized LDL increases. Oxidized glutathione (GSSG) increases. Past degradation of oxidized proteins in lymphocytes causes apoptosis.

TAT (a protein from HIV) has paracrine effects.

In countries where 5-10% of the population is HIV infected, a lifelong treatment, generating side effects and resistant strains of virus, is unsustainable. So instead, we must have *functional* eradication of HIV infection. We must stimulate the patients' own immune systems to wipe out the HIV. We don't know yet exactly how to do this, but this is our goal.

Vaccine against the viral envelope protein.

What is the nature of the reservoir? viral DNA. He says that he can detect resonance emission of low frequency electromagnetic waves by some DNA molecules. 100Hz detects only at certain concentrations in water, 15-100 nM. Perhaps in higher concentrations, the strands aggregate and don't emit a signal.

The origin of the EMS is HIV DNA, also localized in the erythrocyte fraction of blood.

Novel Infectious Agents in Human Carcinogenesis: State and Perspectives

Harald zur Hausen, Deutsches Krebsforschungszentrum, Heidelberg, Germany

(Introduction by *Helmut Sies*)

Notes: Many cancers are caused by various viruses and parasites. Infectious agents are direct carcinogens. 21% of global cancer incidence is caused by infections: 0.8% of that by parasites; 35% of that by bacteria; 65% of that by viruses.

DNA virus families involved in human cancers:

- Herpes, incl human Herpesvirus type 6;
- Human Cytomegalovirus (CMV) linked to brain tumors;
- papilloma,
- Polyomavirus, including JC Polyomavirus linked to colorectal cancer and brain tumors;
- Hepatitis C Flavivirus,
- Retroviruses including HIV;
- Helicobacter pylori

Either as integration of specific viral genes into host DNA or existing as episomal elements. In some cases the viral gene locates next to a host growth gene, and facilitates malignant conversion. Also, infections can induce inflammation, which induces cancer.

Parasites are linked to cancers: Schistosoma Haematobium, Ppisthorchis viverinin.

To discover new infectious etiologies, look at cancers that occur at increased frequencies under immunosuppression. Look for cancers in organ transplant recipients or after HIV infection. On the other hand, Mouse Mammary Tumor Virus (MMTV) has reduced incidence under immunosuppression.

There are difficulties in interpreting mouse cancer studies because chemical carcinogens can release endogenous mouse tumor viruses.

Several open questions remain: Do HSV-2, CMV, or UV amplify existing cancer viruses?

The human JC virus induces tumors in some animals.

In some cases, there are suspicious associations of cancers related to associations with domestic or farm animals. However, early infections and dirty living conditions are protective against childhood leukemias.

Sies: There is a large difference in thiol redox state between differentiating cells and proliferating cells.

Thursday, 18 March 2010

SESSION I WOUND HEALING

In the United States, chronic wounds affect around 6.5 million patients. It is claimed that an excess of US\$25 billion is spent annually on treatment of chronic wounds and the burden is growing rapidly due to increasing health care costs, an aging population, and a sharp rise in the incidence of diabetes and obesity worldwide. Ischemia is one of the most common complications adversely affecting wound healing. The session on wound healing will address current advances in basic sciences and how such sciences are being applied to manage wound

CHAIRPERSONS

Chandan K. Sen, Comprehensive Wound Center, Department of Surgery, Davis Heart & Lung Research Institute, The Ohio State University Medical Center, Columbus, OH, USA

Thomas K. Hunt, Wound Healing Laboratory, University of California, San Francisco, CA, USA

Homing to hypoxia: The role of oxygen tension in progenitor cell trafficking to sites of injury

Geoffrey C. Gurtner, Department of Surgery, Stanford University School of Medicine, California

Notes: Rodent model. With aging, we see an impaired ischemic response in wound healing. Aging impairs new blood vessel growth, but not baseline vascular density. Aging impairs tissue ischemia-induced SDF-1 expression, and other wound healing genes. Aging impairs HIF-1 α stabilization directly and leads to necrosis of tissue. In aging, methyl glyoxalation is responsible for this at Arg354 in the CH1 domain of p300. In diabetes and hyperglycemia, we see a similar event. They found the same patterns of hypoxia response in aged humans and diabetic humans. Diabetes plus aging compounds the problem. Desferoxamine (DFO) chelates iron and salvages impairment of HIF1 α , preventing necrosis, enhancing wound healing and neovascularization.

Ben Treadwell, Juvenon: Cancer increases with age. Are you increasing cancer by increasing neovascularization?

A: Good question. We are using local, topical delivery to minimize systemic risk.

Cross, UC Davis: Metabolic memory?

A: Right. Correcting diabetes by chemical modification does not fix all of these defects.

Kagan, U of Pittsburgh: Could high levels of Vitamin C help with the hydroxylation phase?

A: The next speaker will address this.

Garry Gordon: We have used EDTA for years and saved many extremities. Thank you for your work to explain how the chelation was working.

UC Berkeley: Would you distinguish between ischemia and hypoxia?

A: In these settings, ischemia is predominant, because blood is blocked off for a long time. In exercise, hypoxia is a temporary reduction in oxygen concentration in the tissue. It is demand mediated and temporary.

Hyperbaric oxygen therapy and stem cell response in wound healing

Omaida Velazquez, The Dewitt Daughtry Family Department of Surgery, Leonard M. Miller School of Medicine, University of Miami, Florida, USA

Notes: Diabetic non-healing wounds are a major problem, leading to amputations and limb loss.

Endothelial progenitor cells (EPC) participate in wound healing. They are mostly from bone marrow. EPCs can be extracted, expanded, and reinjected. They are already in phase 2 clinical trials. Hyperbaric oxygen (HBO) increases EPC mobilization. SDF-1 α increases EPC homing. Together, they increase wound healing in diabetic wounds. SDF1 α is chemokine involved in homing of EPC to peripheral tissue and other sites (bone marrow niches). However, it is also found in tumors. SDF-1 α induces adhesion molecules to improve homing of EPCs to EC.

HBO increases NO in bone marrow. In human trials, HBO increases mobilization of EPCs. We need to add local wound therapies to improve homing of EPCs.

Mark Clement, UNC, Charlotte: Remarkable results! Is this activation of eNOS? Do you have ideas of

the mechanisms of activation?

A: We think it's activation of all of the NOSs. We can eliminate the effect by injecting LNAME.

Clement: How does HBO increase NOS activity?

A: We don't know, but we would like to know.

Q: Why do incisional wounds benefit less from this treatment than excisional wounds?

A: EPCs are not needed for incisional healing because it is just *rejoining* blood vessels, but not growing new ones.

Cameron Rink, OSU: Is vasodilation from NO involved in the mechanism?

A: Maybe in peripheral tissue, but unlikely in bone marrow.

Impaired resolution of wounds in diabetes: role of macrophages

Sashwati Roy, Associate Prof, Comprehensive Wound Center, Department of Surgery, Davis Heart & Lung Research Institute, The Ohio State University Medical Center, Columbus, OH, USA

Notes: *Regeneration* is exact replacement. Normal repair is reestablished equilibrium. Excessive healing is fibrosis. Deficient healing causes chronic ulcers. Chronic ulcers is the most common cause of foot amputations.

What is *inflammation*? Inflammation is a local tissue response to injury and a measure to limit infection. Resolution of inflammation usually starts after several hours of inflammation response. *Resolution* involves downregulation of pro-inflammatory mediators, and restoration of normal permeability of blood vessels.

We see many apoptotic cells in diabetic wounds. (TUNEL stain for caspase positive cells). Is it decreased clearance of dead cells by macrophages, or increased numbers of apoptotic cells?

Impaired efferocytosis.

Macrophages recognize cell surface markers and oxidized phosphatidyl serine on apoptotic cells. But in diabetes, there is an impaired oxidase pathway that results in macrophages not recognizing dead cells for clearance.

They vacuumed out fluid and cells from diabetic chronic wounds, and analyzed them. They found high levels of Oncostatin-M (OSM) gene expression. Prostaglandin E2 (PGE2) is mediator of OSM, via EP2 and EP4 receptors ==> G-protein (cAMP).

Kagan, Pittsburgh: You can enhance wound healing by another method with nanoparticles coated with phosphatidyl serine.

A: Yes, we are working with this paradigm, and nanoparticles is a promising method.

Univ of Vermont: Is there evidence for activation of the HIF pathway in wound macrophages?

A: We have not seen it yet, but we might see it in diabetic wound macrophages. We are going through a huge amount of data.

Q: Antiinflammatories are often used to control pain. How does this affect healing?

A: You need inflammation early to clean the wound, but then it is good to stop the inflammatory response.

Mathematical modeling of the role of oxygen in nonischemic and ischemic wound healing

Avner Friedman, Mathematical Bioscience Institute, Ohio State University, Columbus, Ohio

Notes: He is a member of U.S. National Academy of Sciences.

The MBI was founded 8 years ago to bring together mathematicians and biologists to work on biological problems using mathematical techniques. NSF will give it \$16M for the next 5 years.

Treating chronic wounds currently costs \$10 Billion per year.

We want to predict and best schedule application of HBO.

Phases: Clotting, hemostasis. inflammation, cleaning. healing. remodeling.

Fibroblasts produce extracellular matrix (ECM).

They set up a system of 8 partial differential equations involving rates of reactions. They are modeling the role of oxygen in VEGF production.

(Xue C, Friedman A, Sen CK. A Mathematical Model of Ischemic Cutaneous Wounds. PNAS 106, Sep 2009.)

Cell-based therapies for peripheral vascular disease

Douglas Losordo, Feinberg Cardiovascular Research Institute, Northwestern University, Feinberg School of Medicine, Chicago, IL, USA

Notes: They are isolating putative endothelial progenitor cells (EPCs) for angiogenesis. They measured capillary density after myocardial infarct, comparing injection of saline or EPCs or CD34 cells.

Chronic Myocardial Ischemia (CMI) patients ~750,000 in US.

Angina Class 3 = chest pain with moderate activity

Angina Class 4 = chest pain while brushing teeth.

They found significant improvement after 6 months in patients who received a low dose of their own (autologous) CD34 stem cells to their myocardium. They have seen a good safety profile.

Critical Limb Ischemia (CLI). Without this new therapy, 20% of CLI patients die in first year. 30% survive with amputation in first year. Stem cells therapy improved these outcomes significantly.

Q: Do you believe that the injected CD34 cells differentiate into vascular cells in vivo?

A: Yes, but also, they secrete cytokines and recruit additional cells.

Ischemic wound healing

Thomas Mustoe, Division of Plastic and Reconstructive Surgery, Department of Surgery, Northwestern University Feinberg School of Medicine, Chicago, IL, USA

Notes: They use an ischemic rabbit ear model. They found a beneficial synergetic effect of HBO and growth factors. Ischemia impairs the host response to bacterial biofilms. (Human Gene Therapy 17(6)) Aged cells have altered response to oxidative stress.

A novel role for caspase-8 in regulating the cutaneous wound-healing response

Colin Jamora, Cell And Developmental Biology, Division of Biological Sciences, University of California San Diego, La Jolla

Notes: Wounding alters the homeostatic balance between survival and death (toward survival) in cells of the outer layer of skin, as well as the balance between proliferation and differentiation (toward proliferation) in cells of the lower layer of skin. Caspase-8 signaling is involved. More than a dozen other proteases are involved, including several MMPs. IL-1 α induces NF κ B activation in keratinocytes. NALP3 inflammasome is required for IL1a secretion.

Q: What is the role of caspase8 in keratinocyte migration?

Microcirculation in tissue injury and repair

Mark G. Clemens, Department of Biology, University of North Carolina, Charlotte

Notes: Endothelin-1 (ET-1) is a vasoconstrictor, proangiogenic, stimulates proliferation, and increases vascular permeability. Endotoxemia potentiates liver response to ET1.

Stress induces overexpression of caveolin-1 (Cav1) and greater response to ET1. Cav1 downregulates eNOS.

Carbon monoxide (CO) acts as vasodilator. Hydrogen sulfide (H₂S) is the third gaseous mediator. H₂S acts as a vasodilator. It enhances healing of gastric ulcers, and stimulates angiogenesis.

Inflammation upregulates HO1, Cac1, H₂S, and ET1.

Sofi: We find that inhibiting eNOS gives bad effects in horse laminar disease.

A: Yes. The interactions are complex. Almost always, inhibiting eNOS gives bad results.

SESSION II REDOX SIGNALING AND INFLAMMATION

CHAIRPERSONS

Junji Yodoi, Institute for Virus Research, Biological Responses Department, University of Kyoto, Kyoto, Japan

Elias Arnér, Division of Biochemistry, Department of Medical Biochemistry and Biophysics, Karolinska Institute, Stockholm, Sweden

Redox regulation of thioredoxin and glutaredoxin systems

Arne Holmgren, Division of Biochemistry, Department of Medical Biochemistry and Biophysics, Karolinska Institute, Stockholm, Sweden

(Holmgren A, Lu J. Thioredoxin and thioredoxin reductase: current research with special reference to human disease. *Biochem Biophys Res Commun.* 2010 May 21;396(1):120-124.)

Notes: Mammalian ribonucleotide reductase (RNR) enzyme. During replication, the enzyme connects 100,000 nucleotides per second, and forms 100,000 disulfides per second, which have to be reduced.

The p53R2 subunit is involved in the cell-cycle checkpoint. Mutation of p53R2 causes depletion of mtDNA. It appears to coordinate nDNA synthesis with mtDNA synthesis. The p53R2 sequence is 80% similar to R2 sequence.

Glutathione (GSH) is increased in rapidly proliferating cells. Decreased GSH limits cell proliferation. Human Trx1 has 3 more cys residues than mouse Trx. H₂O₂ signaling can reversibly inactivate huTrx by forming an intramolecular disulfide bond.

Humans have several Grx genes.

Inactivation of peroxiredoxin I by tyrosine phosphorylation at lipid rafts allows spatially controlled accumulation of H₂O₂ for intracellular signaling by growth factor or immune receptors

Sue-Goo Rhee, Division of Life and Pharmaceutical Sciences, Ewha Womans University, Seoul, South Korea

Notes: There are 6 isoforms of peroxiredoxin (Prx), abundant in cytoplasm of all types of cells. This talk focuses on Prx 1 and 2. Prx1 is phosphorylated at the edge of a wound during healing. Phosphorylation is done mostly by src kinase when activated by growth factor. (Woo. *Cell* 140 p517-528, 2010).

To serve as an intracellular messenger, H₂O₂ must be protected from destruction by Prx.

Kagin, Univ of Pittsburgh: There are many transition metal-protein complex enzymes.

A: Prx is not very efficient catalyst, but it has an H₂O₂ binding site, so probably main function is to remove H₂O₂.

Thioredoxin and Thioredoxin-Binding Protein-2 (TBP-2) in redox signaling

Junji Yodoi, Institute For Virus Research, Biological Responses Department, University of Kyoto, Japan

Notes: TBP2 modulates lipid metabolism and NKT cell-dependent innate immunity in liver.

Redoxosome complex.

TRX tg mice resist damage from cigarette smoke and diesel exhaust. They resist allergic inflammation and COPD from cigarette smoke. They have reduced mucin production after exposure to cigarette smoke. TRX contributes to influenza virus infection resistance. rTRX quenches the cytokine storm in tg mouse. Food with TRX-extract, derived from sake processing, appears to have some benefit in protecting the mucosal membranes of mice from smoke. Skin resistance of TRX tg mouse is improved.

Cross, UC Davis: How resistant is the TRX tg mouse to invasive organisms injected into abdomen? Is there a cytokine storm? Does it modify bacterial killing?

A: Testing is underway.

Arne Holmgren: What is the ratio of TBP2 and Trx in normal people and normal mice?

A: They appear to be equimolar.

Holmgren: Probably they form a complex with a disulfide bond.

Immunoregulatory role of GIF/MIF, a redox-gearred cytokine

Katsuji Sugie, Division of Developmental Immunology, La Jolla Institute For Allergy and Immunology, La Jolla, CA, USA

Notes: First described in 1960s as GIF, a proinflammatory cytokine. Later rediscovered as MIF, an immunosuppressive cytokine. MIF = Macrophage Migratory Inhibitory Factor.

Cysteinylation of C60 is essential to function. Cysteinylation of GIF is secreted by T cells, involves

ER/Golgi.

Is Prx1 a GIF receptor?

Yodoi: Would Prx1 KO mice work as model for human allergies?

Q: Cysteinylation changes surface characteristics quite a bit, but very different than glutathionylation, which puts a negative charge on a protein.

NADPH oxidases in the lung: beyond host defense

Albert van der Vliet, Department of Pathology, University of Vermont College of Medicine, Burlington

Notes: The airway epithelium is an interface with the external environment. It secretes mucins/defensins for host defense. DUOX1/2 is in mucosal host defense. MMP-9 contributes to the airway epithelium wound response. ATP release is a common epithelial response to bacterial ligands. ATP release stimulates H₂O₂ production and epithelial cell migration.

DUOX1 activation promotes epithelial production of IL8 by bacterial stimuli. DUOX1 activation promotes ADAM17 activation and TGF α release.

How does ATP activate EGFR? Look at EGFR/HER1 signaling.

DUOX appears to be epigenetically silenced in many lung cancer cell lines, but they overexpress other NOXs, so the implications are not clear.

Q: H₂O₂ can act either as oxidant or as a reductant. Did you look at the possibility that it might be acting as a reductant.

A: I have not done that experiment.

Regeneration of infarcted myocardium with nutritionally modified cardiac stem cells: Implication for redox signaling. "The Resveratrol Miracle: a therapeutic promise for alternative medicine"

Dipak K. Das, Cardiovascular Research Center, University of Connecticut School of Medicine, Farmington, Connecticut, USA

Notes: Resveratrol is easy to get from red wine or grapes. It has many beneficial effects. Anti-thrombin activity. It inhibits MAPK activation.

Resveratrol \implies iNOS \implies VEGF \implies eNOS

Resveratrol \implies Adenosine A₃ receptors \implies MEK

Resveratrol at low concentration activates Atg5 and autophagy.

He asks if Resveratrol might perhaps be a hormetic compound, which is effective at low dose, but somewhat toxic at high dose?

Mitochondria, oxidative stress, and cell death

Sten Orrenius, Division of Toxicology, Institute of Environmental Medicine, Karolinska Institute, Stockholm, Sweden

Notes: ROS promote mitochondrial pore transition. Apoptosis Inducing Factor (AIF) is a 62 kDa FAD-containing protein anchored to the IMM. Cleavage of the tether and release of AIF into the cytosol can cause apoptosis. But, AIF KO is embryonic lethal.

HCN1,2,3,4 Hyperpolarization-activated, cyclic nucleotide-gated calcium channels are widely expressed in CNS and myocardium.

Development of high field Overhauser-MRI and its application to in vivo imaging.

Hideo Utsumi, Department of Bio-Functional Science, Faculty of Pharmaceutical Sciences, Kyushu University, Fukuoka, Japan

Notes: OMRI is a double resonance technique, which creates high resolution and 3D images of free radical distributions in small animals. (JCBF 2009) (PNAS 2009). They built it in their lab, but they expect it to be commercialized within this year. OMRI can image the pharmacokinetics of 2 drugs simultaneously. OMRI can image blood vessel permeability. It can image oxygen concentration inside a tumor.

Q: What is the time resolution? How long does it take to get an image?

A: It depends on the magnetic field strength. We build 3 devices. 2 minutes for 1.5T OMRI.

Q: You expose patient to both high magnetic field and high microwave field.

A: That is a very important point. We need new technologies.

Lipoic acid reverses the age-related loss of Nrf2-mediated antioxidant gene transcription

Tory M. Hagen, Ph.D., Linus Pauling Institute, Oregon State University, Corvallis

Notes: (Tory is recovering from his 8th back surgery, so Balz Frei presented his paper.)

We are facing an impending wave of senior citizens in the U.S. 80% of Americans > 65 yrs suffer from at least one chronic disease. Tory has been working on R- α -Lipoic Acid (R-LA). R-LA improves hepatic antioxidant capacity, which otherwise declines with age. It does this by reversing the age-related loss of endogenous antioxidants, especially GSH. Normally, the nuclear level of NF-E2-related-factor-2 (Nrf2), a transcription factor that regulates expression of GSH-synthesizing enzymes, markedly declines with age, but R-LA reverses this loss.

R- α -LA \Rightarrow IRS-1 \Rightarrow PI3K \Rightarrow PI(4,5)P2 \Rightarrow PI3,4,5)P3 \Rightarrow PDK \Rightarrow Akt \Rightarrow Nrf2 \Rightarrow GSH-synthesizing enzymes \Rightarrow increased GSH levels.

Feeding old rats for 2 weeks 0.2% LA reverses the age-related loss of GSH. They resist toxic peroxides just like young rats.

Chromatin immunoprecipitation (ChIP) found lots of Nrf2 binding to the ARE3 site, but not to the other ARE sites. LA induces Nrf2 binding to 2 ARE sites in the GCLC promoter from old rat hepatocytes.

06:30 *Poster Presentations and Refreshments*

Friday, 19 March 2010

SESSION III CARDIOVASCULAR

Cardiovascular disease is a worldwide, major cause of death: this session provides an update of already established and new therapeutic approaches that target the cell redox status and can be used for treating cardiovascular disease with diabetic and atherosclerotic components.

CHAIRPERSONS

Dipak K. Das, Cardiovascular Research Center, University of Connecticut School of Medicine, Farmington, CT, USA

César G. Fraga, Physical Chemistry, School of Pharmacy and Biochemistry, University of Buenos Aires, Argentina

Rescue of diabetes-related impairment of myocardial angiogenesis: potential and challenges

Nilanjana Maulik, University of Connecticut Health Center, Department of Surgery, Molecular Cardiology And Angiogenesis Laboratory, Farmington, CT, USA

Notes: VEGF and eNOS increase angiogenesis, which can help rescue diabetic myocardium. They implemented gene therapy by injecting an adenovirus vector into diabetic myocardium: Thioredoxin-1 (Trx1) is an antioxidant protein. VEGF. They saw significant improvement after myocardial infarction in diabetic rats. (Circulation, 2010 in press) (Diabetes 59:51-60, 2010)

Stockholm: Impressed that they achieved such good effects with such a small increase in Trx.

Tamara Sofi: Stanford is doing work on eNOS for diabetic myopathy. Is there an effect of Trx on eNOS.

A: That remains to be determined.

Q: Is the transfection permanent?

A: We measured the results 30 days after treatment. Results were good at that time.

Inducible nitric oxide-derived nitroso-redox balance as a key modulator of tolerance to ischemia/reperfusion injury in diabetic patients

Hajime Otani, MD, Department of Thoracic And Cardiovascular Surgery, Kansai Medical University, Moriguchi City, Osaka, Japan

Notes: Treatment with tetrahydrobiopterin (BH4), which is a NOS cofactor, increases NOx in the

diabetic heart. Oxidative stress reduces BH4 levels. BH4 therapy replenishes the pool of BH4.
Todd: Diabetes is a reductive stress disease, rather than oxidative stress.

Regulation of myocardial growth and death by oxidative stress

Junichi Sadoshima, Department of Cell Biology And Molecular Medicine, Cardiovascular Research Institute, New Jersey Medical School, University of Medicine And Dentistry, Newark, NJ, USA

Notes: Trx1 suppresses nuclear export of HDAC4.

Redox status regulates class II HDACs and cardiac hypertrophy.

Nox4 tg produces more superoxide in heart cells. Nox4 contains a mito localization signal on its N-terminal region.

Contribution of cholesterol oxidation products to the progression of atherosclerotic lesion

Giuseppe Poli, Department of Clinical And Biological Sciences, University of Turin, San Luigi Gonzaga Hospital, Turin, Italy

Notes: 7-ketocholesterol induces inflammatory cytokines, such as IL-1 β . Oxysterols induce the MEK1/2 pathway, resulting in upregulation of the CD36 receptor. Abnormal patterns of oxysterols exist in brains of Alz patients.

SESSION IV TRANSLATIONAL SCIENCE BY MICRONUTRIENTS

ORGANIZERS **Lester Packer and Enrique Cadenas**, Pharmacology and Pharmaceutical Sciences, School of Pharmacy, University of Southern California, Los Angeles, CA, USA

Considerable advances in translational science by micronutrients underscore the development of specific therapies for human diseases. Some examples for carotenoids and coenzyme Q: identification of cell targets, gene defects, and single gene polymorphisms, SNPs (*e.g.*, for carotene monooxygenases), thus providing the basis for poor bioavailability. Fostering cardiovascular function sets the basis for the health effects of polyphenols, such as resveratrol, along with its anti-aging effects, which partly mimic the gene expression profile of caloric restriction.

CAROTENOIDS AND VITAMIN A

Session Dedicated to the Memory of Norman I. Krinsky

CHAIRPERSONS

Helmut Sies, Institute of Biochemistry and Molecular Biology I, Heinrich-Heine-University, Düsseldorf, Düsseldorf, Germany

Klaus Kraemer, Sight And Life, Basel, Switzerland

Norman I. Krinsky

Helmut Sies

Notes: Norman Krinsky was one of the early organizers of the Oxygen Club of California. He spent 56 years in carotenoid research, on the faculty of Tufts University. He died in 2008.

NORMAN I. KRINSKY MEMORIAL LECTURE

Carotenoid cleavage and biological action: Carotenoids and Vitamin A. (Colors with functions: Elucidating the biochemical and molecular basis of carotenoid metabolism)

Johannes von Lintig, Pharmacology Department, Case Western Reserve University, Cleveland, OH,

Notes: Carotenoids have several functions. Importantly, they are precursors of Vitamin A. Vitamin A has many important physiological functions. Carotenoids include β -carotene, zeaxanthin, lycopene, astaxanthin, and β -cryptoxanthin.

Vitamin A enters cells by passive diffusion, but carotenoid uptake is protein-mediated.

Negative feedback regulation controls Vitamin A production and excess carotenoid absorption to prevent excess vitamin A. The 3-OH group acts as a redox system.

BCDO2 KO (β -carotene 15,15'-monooxygenase) mice accumulate dietary carotenoids in their livers.

Excess carotenoid supplementation might be harmful.

β -Carotene dioxygenase-1 polymorphism

Georg Lietz, Human Nutrition Research Centre, School of Agriculture, Food and Rural Development, Newcastle University, Newcastle Upon Tyne, UK

Notes: After absorption, pro-vitamin A carotenoids are converted to retinal by the enzyme β -carotene 15,15'-monooxygenase (BCMO1).

A new Micronutrients Genomics Project aims to build a bioinformatics portal. Contact Georg to get involved: Georg.lietz@ncl.ac.uk

The β -carotene 15,15'-monooxygenase-1 gene affects circulating levels of carotenoids

Richard D. Semba, Department of Ophthalmology, Johns Hopkins University School of Medicine, Baltimore, Maryland

Determination of 9-cis β -carotene and ζ -carotene in biological samples

Guangwen Tang, Dorothy J. And Gerald R. Friedman School of Nutrition Science And Policy, Tufts University, Boston, Massachusetts

Control of oxidative phosphorylation by vitamin A illuminates a fundamental role in mitochondrial energy homeostasis

Ulrich Hammerling, Memorial Sloan-Kettering Cancer Center, Immunology Program, New York, NY

Public health aspects of β -carotene as an important vitamin A source for humans

Hans K. Biesalski, Institute for Biochemistry and Nutrition Science, University of Stuttgart-Hohenheim, Germany

Notes: The conversion of β -carotene to vitamin A differs quite a lot among different people. Liver is the most significant dietary source of pre-formed vitamin A.

Lester Packer: There is now a portable laser scanner that can assess carotenoid status of people by scanning their skin.

COENZYME Q

CHAIRPERSONS

Maret G. Traber, Linus Pauling Institute, Oregon State University, Corvallis, Oregon, USA

Roland Stocker, Centre For Vascular Research, School of Medical Sciences, University of New South Wales, Sydney, New South Wales, Australia

New classes of mitochondrion-targeted antioxidants – non-radical scavenging inhibitors of lipid peroxidation

Valerian Kagan, Center For Free Radical And Antioxidant Health And Department of Environmental And Occupational Health, University of Pittsburgh, Pittsburgh, PA, USA

Notes: Long ago, he worked in Lester Packer's lab at UC Berkeley on antioxidant recycling. Ubiquinol can regenerate vitamin E. Some phospholipids are "2-legged"; others are "4-legged". There are 12 possible lipid chains on each leg. Cardiolipin is "4-legged", with 2 negative charges. They use LC-MS to distinguish the variations. They look at the spectrum of oxidized cardiolipin to see an oxidized lipidomics analysis after γ -irradiation of mouse lung.

The triphenylphosphonium (TPP) group can be used to target chemicals into the mito. TPP-conjugated nitroxides are protective against radiation damage-induced peroxidation. He believes that cardiolipin oxidation products are interacting with other biomolecules and causing problems.

Coenzyme Q in plasma membrane electron transport

Plácido Navas, Centro Andaluz De Biología Del Desarrollo, Universidad Pablo De Olavide-Csic, Sevilla,

Spain

Notes: CoEnzymeQ₁₀ (CoQ₁₀) was discovered by Fred Crane in 1954. It has many functions in the cell, including β -oxidation, uncoupling protein, antioxidant, and more.

(Villalba. PNAS, 1995) (Navas. Mitochondrion, 2007) Trans-plasma-membrane electron transport system. With increasing age, we see a decline in the concentration of a critical enzyme that makes CoQ. NQR1 is induced by CR. NQR1 increases respiration and decreases fermentation.

(de Cabo. Aging, 2009)

Ecto NOX, coenzyme Q10, and superoxide production in aging

D. James Morré, Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, Indiana, USA

Notes: arNOX, 28-30 kDa protein, has negative effects. arNOX is found in sera, saliva, and sweat of people over age 30-50. arNOX activity of saliva and sera correlate tightly. It is complexed with apolipoprotein B (apoB). He wants to find inhibitors of arNOX. CoQ₁₀ is one such inhibitor. The human response to oral CoQ₁₀ is maximal from 2 to 8 hours after ingestion. arNOX takes electrons from dithiol apoB protein to make 2 superoxides from O₂, so it is involved in LDL oxidation.

Lester Packer: Many activities go down with age. This system goes up with age and produces superoxide.

A: arNOX might have benefits while associated with each cell. It's bad effects arise when it leaves the cell and becomes associated with LDL, and oxidizes it.

Coenzyme Q₁₀ in health and disease

Iain P. Hargreaves, Department of Molecular Neuroscience, Institute of Neurology, Queen Square, London, UK

Notes: CoQ₁₀ acts as electron carrier and as a lipophilic antioxidant. CoQ₁₀ protects cell membranes against free radical induced oxidation.

Clinical assessment of CoQ₁₀ status is usually measured by measuring blood plasma, but does it accurately assess muscle status? His lab now measures blood monocytes and lymphocytes.

LDL carries 60% of circulatory CoQ₁₀.

Few patients have side effects from statin treatment, but it is serious to muscles. Side effects from statin treatment can be more common if they are also on other drugs such as itraconazole or cyclosporin.

Cross, UC Davis: In vitamin E, we just correct for the difference between plasma and cellular levels.

A: Plasma reflects liver synthesis as well as dietary intake.

POLYPHENOLS

CHAIRPERSONS

Balz Frei, Linus Pauling Institute, Oregon State University, Corvallis, OR, USA

Manfred Eggersdorfer, DSM, Basel, Switzerland

Targeting inflammatory pathways 'naturally' for prevention and therapy of cancer and other chronic diseases

Bharat B. Aggarwal, Department of Experimental Therapeutics, The University of Texas M.D. Anderson Cancer Center, Houston, Texas, USA

Notes: 30% of all cancers are due to tobacco. 50% are due to bad diet and obesity. 5-10% are due to genetic background.

Prostate cancer incidence is 2 per 100,000 for Chinese in China, but 23 per 100,000 if they have been in the USA for more than 5 years. Prostate cancer incidence for Caucasians born in USA is 58 per 100,000. Inflammation is a risk factor for most cancers. NF κ B is the major mediator of inflammation. As long as NF κ B and TNF remain in the immune system, then are probably good for you; it is when they get out that they cause cancer.

They did study of ayurvedic plant therapies. Nutraceuticals are less likely to be toxic than targeted drugs

because they have low affinity and target multiple genes to dial them down just a little, while drugs target a single gene with high affinity.

Many plant products block NF κ B, including diferuloylmethane (curcumin from turmeric), katuka, goldenseal, pinitol talisapatak, resveratrol, Indol-3-carbinol (from broccoli), tocotrienol (from coconut), (but not tocopherol), spices, onion, fennel, rosemary, red chili, ginger zerumbone, black cumin, fenugreek, and fennel (Aggarwal. Exp Biol & Med 2009).

Treadwell: of all the compounds, which do you think is the most exciting for cancer inhibition?

A: More science has been done on curcumin, so it is the most exciting so far.

Chemoprevention by pomegranate and green tea

Susanne M. Henning, Center For Human Nutrition, David Geffen School of Medicine, UCLA, Los Angeles, CA, USA

Notes: Pomegranate metabolizes into: ellagic acid, dimethyls EA, Urolithin A and B.

Tea polyphenols are EGCG, EGC and ECG

Pomegranate: Drinking one cup of pomegranate juice per day slows the increase in PSA in men.

Some beneficial compounds are in the pomegranate skin. (Seeram NP. J Nutr 136:2481-2485, 2006) (Seeram NP. J Med Food 11(2):390, 2008)

Ellagitannins (ET) are also in walnut, persimmon, strawberry, and other berries.

Aromatase inhibitors (Adams LS. Cancer Prev Res 3:108-113, 2010)

EA and Urolithins inhibit *Wnt* signaling in colon cancer cells. (Sharma M. J Agric Food Chem 2009)

Green Tea: Drinking brewed green tea inhibits xenograft tumor growth in mice.

JDF: Did your slide show active ingredients in the skin of the pomegranates? Should we eat the skin?

A: Yes. Eating the skin and the connective tissue is beneficial. The commercial juices and extracts squeeze the whole fruit, so you also get these benefits from the commercial juices and extracts.

Regulation of antioxidant gene expression by selected dietary polyphenols

Young-Joon Surh, National Research Laboratory of Molecular Carcinogenesis and Chemoprevention, College of Pharmacy, Seoul, South Korea

Notes:

(Book: Plant Phenolics and Human Health. Ed by CG Fraga. Wiley, 2010)

(Resveratrol suppresses growth of human ovarian cancer cells.)

Resveratrol is found in mulberry, grapes, and red wine.

Oxidative stress can activate Nrf2, either by oxidizing the 2 Cys thiols on Keap1 or by phosphorylating Nrf2 via MAPK. Curcumin and other beneficial compounds are electrophiles, which brings them to the Keap1-Nrf2 complex.

Garlic diallyl sulfide protects against *H. pylori*.

(Surh YJ. Redox-sensitive transcription factors as prime targets for antiinflammatory and antioxidant phytochemicals)

Resveratrol mimics caloric restriction and retards aging in the heart

Tomas A. Prolla, Departments of Genetics & Medical Genetics, University of Wisconsin, Madison

Notes: He is also co-founder of LifeGen Technologies with Rick Weindruck.

How does calorie restriction (CR) extend lifespan?

Age-related hearing loss is halted by CR.

Is Resveratrol (R) a CR mimetic? Does R mimic some of the effects of CR? In some studies, R activates Sirt1; in others, it doesn't. Some studies say R extends yeast lifespan; others say it doesn't. One study said R extends lifespan of fat-fed mice, but not of normal-fed mice.

Prolla started mouse dietary interventions at middle-age (14 months). Mice receive 50 mg resveratrol /kg body weight/day. This is less than some studies by Sinclair, et al.

The resveratrol diet does not cause weight loss, while CR does.

They analyzed gene expression using the Affymetrix MOE430 2.0 array for cardiac gene exp.

<http://www.plosone.org/doi/pone.0002264>

In muscle, of the 1164 genes changed by CR and resveratrol alone, all were in the same direction.

Hypoth: Resveratrol induces Sirt1, which deacetylates PGC1 α , inducing it and its transcriptional targets.

CR is known to induce PGC1 α in mice and humans.

Prolla now believes that resveratrol does not activate Pgc1 α .

CR effects on AKT are not seen with resveratrol alone.

(Chen D. Tissue specific regulation of SIRT1 by CR.) Activation of SIRT1 in liver is **reduced** by CR and increased by high fat diet. **More Sirt1 is not necessarily better. Increased Sirt1 expression is associated with prostate cancer cells.** (JBC 2009)

Bruce Ames: Lipoic acid and resveratrol are both very effective at inducing phase 2 enzymes.

Q: Does exercise training mimic CR?

A: **Exercise is as effective as CR at changing gene expression in cardiac tissue.**

U of Newcastle: Bioavailability of resveratrol is a problem. It goes in rapidly and out rapidly.

A: We did not look at resveratrol levels in tissues. The mice have free access to food with resveratrol around the clock, so their body levels are probably consistent.

Q: Your group did a study on a mixture of resveratrol with quercetin vs resveratrol alone. R+Q activated more genes than R alone.

07:30 *Concert, Gala Dinner, and Prizes and Awards*

CONCERT celebrating the 200th Anniversary of Frédéric Chopin
PRIZES AND AWARDS

Oxygen Club of California & Jarrow Formulas Health Sciences Prize

Young- and Established Investigator Awards

The Science and Humanity Award

Antioxidant & Redox Signaling Translational Research Award

DSM Nutraceutical Research Award

Linus Pauling Institute Awards

Oxygen Club of California Award

Saturday, 20 March 2010

SESSION V REDOX IMAGING

Imaging biosciences provide a unique means for the development of translational redox sciences. This session highlights applications and new methodologies for *in vivo* monitoring of oxygenation, free radicals, and redox status.

CHAIRPERSONS

Periannan Kuppusamy, Division of Cardiovascular Medicine, Davis Heart And Lung Research Institute, The Ohio State University, Columbus, OH, USA

Harold M. Swartz, Center For the Evaluative Clinical Sciences at Dartmouth, Dartmouth Medical School, Lebanon, NH, USA

Development of PET/SPECT probes and their application to *in vivo* molecular imaging

Hideo Saji, Department of Patho-Functional Bioanalysis, Graduate School of Pharmaceutical Sciences, Kyoto University, Kyoto, Japan

Notes: Rapidly growing tumors are usually low in oxygen. Today he will talk about hypoxia imaging agent. Hypoxia-inducible factor-1 (HIF-1) is a transcription factor with an oxygen sensitive α subunit, and a constitutive β subunit.

Protein transduction domain = PTD. POS = PTD-ODD-SAV

Radiolabeled POS: Radionuclide probes for nuclear molecular imaging emit a signal that is picked up by the imaging detector. IPOS = PTD-ODD-SAV-IBB IBB is a biotin derivative. Label IBB with I123/125. Inject radiolabeled IPOS into mouse and see image signal of tumor because of difference in HIF expression.

Application of POS for PET imaging: F¹⁸-POS is not fast enough for the half life of F¹⁸.

Cross, UC Davis: What is the advantage of your system over existing glucose detection?

A: PET sensitivity is not so high.

Cross: Can you link a tumor treating agent to your ligand.

A: (He does not understand the question.)

Q: You should have a negative control, because all tumors have leaky capillaries.

Clinical (EPR) oxymetry for improving diagnosis and treatment in ischemic diseases and tumors and wound healing

Harold M. Swartz, Center For the Evaluative Clinical Sciences at Dartmouth, Dartmouth Medical School, Lebanon, NH, USA

Notes: 4 themes: 2 are old. 1. Concentration of oxygen is important.

2. We need to look at techniques that will tell us how much oxygen is in the area of interest.

3. Electron Paramagnetic Resonance (EPR) is a nice technique that is making it into clinical studies.

4. They are looking for collaborators.

EPR is a magnetic resonance (MR) technique that is affected by oxygen. The field is much smaller than MRI, ~430 Gs vs 15,000 Gs. EPR uses non-resonant absorption of microwaves. Acquisition time is about 30 seconds. For oxymetry, they use India ink, which is already approved for use in humans. They are measuring oxygen in tumors. They can measure peripheral circulation, such as in feet. When measuring feet, must keep temperature at 37 deg to get accurate measurement. The technique is useful for making repeated measurements.

Garry Gordon: It would be good to correlate these measurement with other techniques.

A: Yes, we have done a lot of that which I did not talk about.

Imaging of tissue oxygenation: Novel probes and opportunities

Periannan Kuppusamy, Division of Cardiovascular Medicine, Davis Heart And Lung Research Institute, The Ohio State University, Columbus, OH, USA

Notes: Can be used to visualize tumors, and to see effects of radiation on oxygenation of tumors.

In stem cell therapy, often most of the stem cells don't survive.

SESSION VI AGING

CHAIRPERSONS

Bruce N. Ames, Nutrition and Metabolism Center, Children's Hospital Oakland Research Institute, Oakland, CA, USA

José Viña, Department of Physiology, University of Valencia, Valencia, Spain

Ras-Grf1 deficiency delays aging in mice by mimicking caloric restriction

José (Pepe) Viña, Department of Physiology, University of Valencia, Valencia, Spain

Notes:

1. Longevity associated genes (LAG). How to identify new ones. What physiological manipulations can upregulate LAGs?

2. Estrogenic compounds modulate antioxidant LAG. Female rats have a double dose of estrogen genes compared with males.

3. p53/P16arf

4. Ras/Grf1 KO mice, a new LAG. Guanine Releasing Factor (**GRF1**) KO mice live longer without tumors. 16S rRNA is a biomarker of aging. They test motor coordinate in rats by having them walk a tightrope. GRF1 KO have better coordination. They overexpress cytochrome c oxidase in liver. This probably lowers ROS and lowers oxidized proteins. They have high LV2 metabolic analysis. After a night of fasting, they still have glycogen, compared with wt, who have no glycogen left. They have less IGF1 than controls. They express twice as much Sirtuins as controls.

Manual Serrano works on tumor suppression. Maria Blasco works on telomeres and telomerase.

Old s-Arf/p53 mice have lower levels of oxidized proteins compared with wild type.
Super p53/p16arf/telomerase mice live much longer, even though they do not have any more protection against oxidative stress.

5. Translational research

Are these same genes, found in mice, also found in human centenarians?

RETICEF: Spanish network on aging and frailty. Alzira (Valencia) Centenarian Study. There are 84 centenarians out of a population of 200,000. 83 of them enrolled in the study. LAGs in centenarians.

5.2 Estrogen replacement study. The replacement must begin during or shortly after loss of natural estrogen. Must begin within 3 weeks in mice or rats.

5.3 Exercise: training and exhaustion. Modulation of LAGs by nutrition and exercise. Exhaustion is not good for you. Exhaustion causes oxidative stress.

Longevity and runners in the Tour de France: The runners live longer than the general population. But they are exhausted every week for at least 10 years. But they are fit.

Q: The Tour de France participants are not typical of the general population; they are exceptional.

Tilman Grune: Did KO mice have weight gain or delay in reaching sexual maturity?

A: They are leaner, with same weight, while eating same as controls. They have no delay in reproduction.

Helmut Sies: You plotted GSSG/GSH and Lactate/Pyruvate ratios. What do you conclude about relationship between those ratios?

A: That is an old slide from 1992. I thought it would relate to exhaustion. But there is no relationship between the two ratios.

Lester Packer: We should establish standard methods for measuring centenarians.

A: I agree.

Q: Athletes recover faster than nonathletes. So measurements during exercise are very different than measurements during rest of the day.

Dean Jones: We can now use standardized methods, such as mass spec developed by Bruce Ames. But samples must be collected in a standard way.

Selective autophagy in the Cellular Response to Stress

Ana Maria Cuervo, MD, PhD, Department of Developmental and Molecular Biology, Marion Bessin Liver Research Center and Institute For Aging Studies, Albert Einstein College of Medicine, Bronx, NY, USA

Notes: Ana Maria is from Valencia. Pepe *Viña* was her Professor from medical school.

Cells can perform 3 kinds of autophagy (apgy): macroautophagy (MA), microautophagy, and chaperone-mediated autophagy (CMA). During starvation, the cell obtains proteins by autophagy. But autophagy is also a mechanism of quality control. If the autophagy function fails, then the cell can die. Degradation of lipids by autophagy (Susmita Kaushik) can provide chemical energy to the cell. Triglycerides => fatty acids => β -oxidation => energy. So blockage of macroautophagy (by blocking Atg7) results in an increase in lipid droplets in the cell. They call it "Macrolipophagy". (Singh Nature 2009) (Koga FASEB 2010).

Failure of Macroautophagy:

Insulin and mTOR are negative regulators of Atg, so they block autophagy.

Failure of macroautophagy can cause failure to recognize and turn over dysfunctional mitochondria.

In Huntington's Disease (HD), autophagic vacuoles are empty. (D. Sulzer). HD patients' cells have a lot more lipid droplets. Mitos in HD

We see altered macroautophagy (MA) in Alz Disease. Autophagic vesicles (AV) accumulate in affected neurons. We see a defect in the pH of the lysosomes, so autophagized mitos fuse with lysosomes, but they do not degrade. (Lee, Cuervo, Nixon, et al Cell 141, 1146–1158, June 25, 2010)

We must identify compounds that can reactivate autophagy and resolve the problem.

CMA substrates have a KFERQ motif, which is recognized by chaperones in the cytosol, to transport substrates to the Lamp2a receptor in the membrane of the lysosome. CMA declines with age in normal

mice.

Restoration of CMA with Tet-off tg mouse (Judy Zhang in Cuervo lab). These tg mice have improved cellular homeostasis in the liver at old age. It looks like a young liver.

Ubiquitinylation brings protein aggregates to the lysosome. The ER is healthier looking in the tg mice. Chaperones that are responsible for protein folding in the ER can be turned over by this CMA pathway, so the whole cell is healthier.

Mark Czaja (Albert Einstein) collaborated on lipid recycling.

Treadwell: Since autophagy increases with CR, have you looked at Lamp2a in CR animals?

A: That will be published soon. CR increases basal activity 4 times. Lamp2a is upregulated, but this is not transcriptional upregulation, so it doesn't show up in RNA microarrays.

Kelvin Davies: There are many kinds of proteosomes, as well as CMA. How does the cell decide which pathway to use for which degradation?

A: The cell does not need a limiting decision. There is overlap in pathways. The proteosomes are very fast. Different compartments may have different half-lives. We are using reporters to analyze this now.

Regulation of proteasome-mediated protein degradation during aging: Role of Lipofuscin in Aging.

Tilman Grune, Institute of Biological Chemistry and Nutrition, University Hohenheim, Stuttgart, Germany

Notes: (Jung ABB 2007) Oxidized proteins are a group of various chemicals. Early work was done by Kelvin Davies, Earl Stadtman, Tilman Grune. If unfolded proteins stay very long in the cell, they form initial aggregates. After time, they crosslink and form lipofuscin (LF). They can fill up to 70% of the volume of some (nondividing) neurons in elderly people.

How are intracellular AGEs degraded? The proteasome cannot degrade any of them. Oxidized proteins are easier for the proteasome to degrade, but not AGE-proteins. Cathepsin D and Cathepsin B are lysosomal digestive enzymes, which are the best at degrading AGE-modified proteins, such as methyl glyoxal.

Vimentin is an AGE-modification target. CML induces redistribution of Vimentin. When there is a modified protein that the cell cannot degrade immediately, the cell tries to sequester it in a lysosome. Chronic oxidation stress leads to nuclear protein oxidation and Stress Induced Premature Senescence (SIPS).

LF and lysosomes: Aggregates are transported into the lysosome by macroautophagy. LF in lysosomes produces ROS, which make more LF. Protein Aggregates and LF inhibit proteasomes. Part of the inhibition is due to oxidation.

LF and the MMP1/TIMP1 ratio: Could LF be regulating these genes?

Lactacystin treatment knocks down proteasome activity and up MMP1 and down TIMP.

Q: How does lysosome produce ROS?

A: If the lysosome is loaded with LF, it contains redox active metals, and it will produce ROS.

Lysosomal membranes have higher vitamin E content than other membranes in the cell, probably to protect them from oxidative stress.

Q: Cathepsin has an Estrogen Response Element in its promoter.

A: Probably nutritional modifications are important.

Delaying age-related disease with micronutrients: Triage theory

Bruce N. Ames, Nutrition And Metabolism Center, Children's Hospital Oakland Research Institute, Oakland, CA, and Professor, University of California, Berkeley, USA

Notes: Metabolism is very complicated. We require about 40 micronutrients, including amino acids, vitamins, 15 minerals, 2 essential fatty acids (EFAs). A moderate deficiency ages you somewhat.

Committees set the EAR. Many American are below the EAR for several micronutrients.

Mg is found in chlorophyll. 56% of Americans are low in Mg. 12% of Americans are low in Zn. 93% of Americans are low in vitamin E. 49% of Americans are low in vitamin B6.

Even though half of Americans take multivitamins, they do not have enough Ca or fish oil because the

pill would be too big. Depletions of any of the micronutrients causes DNA damage.

Jim T. MacGregor is a cytobiologist. (Everson RB, MacGregor JT. J Natl Cancer Inst 80:525-529, 1988) Micronuclei in RNA positive erythrocytes vs RNA negative erythrocytes. Depletion of folic acid causes chromosome breaks similar to radiation. The spleen removes RBCs with stiff membranes. They looked at splenectomy patients.

In the cell, folate is all in the form of reduced tetrahydrofolate (THF). If you don't have enough folate, then homocysteine accumulates, and that is a risk factor for heart disease. 40% of northern Europeans have a polymorphism that gives more heart disease although it pleiotropically protects sperm when there is not enough chlorophyll in the diet.

Base excision repair (BER) processing of opposed lesions can form a dsDNA break, which is the worst kind of DNA damage.

(Fenech 2003 Nutrition Res Reviews) Exposure to X rays vs nutrition.

IRON: Too much iron or too little iron is harmful. Men eating too much red meat get too much iron.

Many women and children do not get enough iron.

Hani Atamna is working on heme synthesis. Complex IV is the first mitochondrial complex to go down when you are short of any substrates. Then mitochondria make more ROS.

Cells low in Zn have more DNA damage and produce more ROS.

Biotin deficiency accelerates cell senescence.

Mg deficiency causes DNA cross links. (Killilea PNAS 105:5768-5773, 2008) The human body wants to keep a ratio of 2 Ca/1 Mg. Deficiencies in just about any micronutrient cause DNA damage, incl Choline, Niacin, Omega-3 fatty acids, Se, B12, K, Zn, Mg, Vit D, Folate, Ca. (Ames. Triage Theory. PNAS 103:17589-594 Nov 2006)

Vitamin K is a quinone used in plants for photosynthesis. Animals use it for blood coagulation. Lack of vitamin K causes calcification of arteries. When we eat greens, vitamin K goes to the liver. The liver converts it to NK, and ships it out to nonhepatic tissues.

Natto from Japan is fermented soybean. It is an acquired taste. It has NK7, which goes to all the tissues. People who eat natto have almost no heart disease.

Immune risk phenotype of aging: Lower CD4 and CD8.

They have developed a food supplement, the **CHORI Bar**, which is complete and more convenient than supplement pills.

Sofi: Supplementing coumadin patients with vitamin K is good for them. What about synthetic vs natural folic acid?

Ames: Synthetic vs. Natural doesn't matter for most micronutrients, except for 2. Oxidized folic acid is used in most pills because it is more stable easier to make. We should use THF.

Endothelial dysfunction in aged humans is related to oxidative stress and vascular inflammation. A practical approach from translational research

Leo Rodriguez Mañas, Department of Geriatrics, Getafe University Hospital, Madrid, Spain

Notes:

1. Normal Aging and the vascular system. Vascular disease is the main cause of death in old people.
2. The CV risk factors and the aged CV system
3. Expression of CVD in old people: Line in adults?
4. Changing our minds.

Glycosylated Hb from old donors induces inflammation

Sofi: Impaired eNOS is a key to endothelial dysfunction.

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Tokiwa Pharmaceutical
The Ellison Medical Foundation
The Ohio State University Medical Center
TRDRP (California Tobacco Research Disease Related Program)
USANA Health Sciences
University of Southern California School of Pharmacy

Abbreviations

aa = amino acids. ab = antibodies.
AD = Alzheimer's Disease. Alz = Alzheimer's.
Abeta = A β = amyloid beta. apop = apoptosis. Apgy = autophagy
C.e. = C.elegans = nematode = worm.
CR = calorie restriction = DR = dietary restriction
cv = cardiovascular. cvd = cardiovascular disease
dsDNA=double-stranded DNA; ssDNA = single-stranded DNA; nDNA=nuclear DNA;
mtDNA=mitochondrial DNA
Dros = Drosophila melanogaster = fly. exp = gene expression.
fn = function GSH = Glutathione (reduced) GSSG = Glutathione (oxidized)
Hb = hemoglobin, the oxygen-carrying protein in RBCs.
HBO = Hyperbaric oxygen (greater than atmospheric pressure)
HSC = hematopoietic (blood-forming) stem cell
IGF = insulin-like growth factor
iPSC = iPS cells = iPC = induced pluripotent stem cells
life ext = lifespan extension.
miR = miRNA = microRNA
mito = mitochondrion mt = mitochondrial mtDNA = mitochondrial DNA.
MSC = mesenchymal stem cells
phosylate = phosphorylate = covalently bind a phosphate group to a molecule
RBC = red blood cell = erythrocyte
ROS = reactive oxygen species or free radicals
Tase = telomerase Tmere = telomere
TLN = translation of RNA to protein. TXN= transcription of DNA to RNA.
vs = versus, compared with, against
w = with wo = w/o = without.
+/+ = homozygous normal gene. KO = gene knockout. KD = gene knock down.
-/- = homozygous KO. +/- = heterozygous gene.
8-oxo-dG = 8OHdG = marker of oxidized DNA.
In biochemical or genetic pathways:
blockage or inhibition --| Activation or causation ==>
Q: or Name: question or comment from the audience.
GMM = George M. Martin
A: answer from the speaker
[JDF: Editorial comments by John Furber.]