SESSION 12
SELENIUM AND HUMAN HEALTH III

ROLE OF Sep15 KNOCKDOWN IN ANIMAL MODELS OF COLON CANCER

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Evidence suggests that selenium has cancer preventive properties that are largely mediated through selenoproteins. Our previous observations demonstrated that targeted downregulation of the 15 kDa selenoprotein (Sep15) in murine colon cancer cells resulted in the reversal of the cancer phenotype. The present study investigated the effect of Sep15 knockout (KO) in mice using two models of chemically-induced colon cancer. In the first model, homozygous Sep15 KO mice, and wild type littermate controls were given azoxymethane. Sep15 KO mice developed significantly ($p < 0.001$) fewer aberrant crypt foci than controls. Dietary selenium above adequate levels did not significantly affect aberrant crypt foci formation in Sep15 KO mice. To investigate molecular targets affected by loss of Sep15, gene expression patterns in colonic mucosal cells of KO and wild type mice were examined using microarray analysis. Subsequent analyses verified that guanylate binding protein-1 (GBP-1) mRNA and protein expression were strongly upregulated in Sep15 KO mice. GBP-1, which is expressed in response to interferon-γ, is considered to be an activation marker during inflammatory diseases, and up-regulation of GBP-1 in humans has been associated with a highly significant, increased five-year survival rate in colorectal cancer patients. In agreement with these studies, we observed a higher level of interferon-γ in plasma of Sep15 KO mice. In the second model, we are currently investigating the role of Sep15 KO and dietary selenium on serum and tissue markers of inflammation and tumor formation in a dextran sulfate salt/azoxymethane model of inflammation-induced colon cancer. Overall, our results demonstrate that Sep15 KO mice are protected against chemically-induced aberrant crypt foci formation and that Sep15 appears to have oncogenic properties in colon carcinogenesis *in vivo*.
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SELENOPROTEINS IN THE THYROID

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Enzymatic production of hydrogen peroxide is required for regular thyroid hormone biosynthesis. Thyroid epithelial cells therefore express antioxidative enzymes, including catalase, peroxiredoxins, thioredoxin reductases, and glutathione peroxidases (GPxs). The latter two enzyme families contain highly active peroxide-degrading enzymes that carry selenocysteine (Sec) in their active centers. Since low Se status has been associated with thyroid disorders, selenoproteins are considered essential for thyroid integrity and function. We have tested the hypothesis that selenium (Se)-containing antioxidative enzymes protect thyroid epithelial cells from oxidative damage by conditional inactivation of selenoprotein biosynthesis in thyrocytes by targeting Sec tRNA.Constitutive and inducible Cre/loxP-mediated recombination of tRNA[Ser]Sec drastically reduced activities of selenoenzymes GPx and type I-deiodinase in thyroid extracts. Immunohistochemical staining revealed increased 4-hydroxynonenal and 3-nitro-tyrosine levels consistent with increased oxidative stress. However, gross thyroid morphology remained intact for at least 6 months after recombination. Circulating thyroid hormone levels remained normal in mutant mice, while thyrotropin (TSH) level was moderately elevated. Challenging mutant mice with low iodine diet increased TSH, but did not lead to destruction of selenoprotein-deficient thyroids.We conclude that selenoproteins protect thyrocytes from oxidative damage and modulate thyroid hormone biosynthesis, but are not essential for thyrocyte survival.

SELENOMETHIONINE AMELIORATES COGNITIVE DECLINE AND REDUCES TAU HYPERPHOSPHORYLATION IN THE TRIPLE TRANSGENIC MOUSE MODEL OF ALZHEIMER'S DISEASE

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Disruption of intracellular balance between free radicals and the antioxidant system is a prominent and early feature in the neuropathology of Alzheimer's disease. Selenium (Se), a vital trace element with known antioxidant potential, has been reported to have the ability to protect the brain from oxidative damage in various models of neurodegenerative disease. In the present study we investigated the therapeutic effect of selenomethione on cognitive dysfunction and neuropathology in the triple transgenic Alzheimer mouse model (3×Tg-AD). 3×Tg-AD mice were treated with selenomethione at the age of 4 and 8 months for 3 months. At the end of treatment, learning and memory of the mice were measured with the Morris Water Maze.

Amyloid and tau neuropathology and biomarkers for synaptic deficit and inflammation were examined in the brain using immunoblotting or immunohistochemistry. Selenomethionine treatment for 3 months significantly improved the spatial learning and memory deficits in 3×Tg-AD mice compared with the control group. Selenomethione treatment significantly reduced total tau and phosphorylated tau in the hippocampus and cortex in the 3xTg-AD mice. The decrease of synaptic protein synaptophysin and postsynaptic density-95 (PSD-95) in the hippocampus and cortex in 3×Tg-AD mice were significantly mitigated by selenomethionine treatment, and astroglial activation in 3×Tg-AD mice was also reduced compared with the control treatment. Additionally, the expression of glycogen synthase kinase 3-β, an important kinases involved in tau phosphorylation, was markedly decreased by selenomethionine treatment. Our results suggest that selenomethionine significantly improves cognition in a murine model of Alzheimer's disease and is associated with reduction in tau and tau hyperphosphorylation, amelioration of inflammation and restoration of synaptic protein.

This study provides a novel therapeutic approach for the prevention of Alzheimer's disease.

MURINE MODELS OF ALTERED Se TRANSPORT HIGHLIGHT BONE AS A PRIVILEGED Se TARGET TISSUE

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Several lines of evidence point to an important role of Selenium (Se) and selenoproteins for bone growth, development and turnover: i) Kashin-Beck disease can be prevented by Se supplementation, ii) Se or selenoprotein P (Sepp) deficiency causes growth retardation in rodents, iii) children with SBP2 mutation display growth retardation, iv) circulating Se and SEPP levels are associated with bone turnover and mineral density in humans. In order to better define the role of Se and selenoproteins in bone physiology, we have compared female and male Sepp-wildtype, -heterozygous and -knockout mice together with SEPP-transgenic mice. Se was determined by total reflection X-ray fluorescence, transcript levels were analyzed by qRT-PCR and proteins were quantified by Western blot. In bone, Se was exclusively associated with proteins but not with inorganic matrix. Upon reduced Sepp expression bone Se decreased and was partially rescued by a hepatic SEPP transgene. Among the set of 24 murine selenoprotein genes, 22 were detected by qRT-PCR with characteristic sex-specific and Se status-dependent differences. Interestingly, Sepp-receptor Apoer2 (Lrp8) was strongly expressed and transcripts were up-regulated in Sepp deficiency indicating a potential feedback loop controlling Se supply to bone in times of poor Se supply. When serum and bone were compared between the different genotypes, Se concentrations differed by up to 25-fold in serum but only up to 2.5-fold in bone. This finding supports the notion that bone belongs to the preferentially supplied tissues within the hierarchy of Se target organs. Our results highlight important sex-specific differences in bone selenoprotein expression, indicate a novel mechanism for preferential Se uptake and contribute to our understanding of the consistent bone phenotype in subjects with SBP2 mutations.Supported by the Berlin-Brandenburg School for Regenerative Therapies (BSRT) and DFG.

SELENOPROTEIN EXPRESSION IN MACROPHAGES IS ESSENTIAL FOR THE RESOLUTION OF INFLAMMATION IN DSS-INDUCED COLITIS

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Ulcerative colitis (UC) is characterized by chronic and relapsing inflammation common in many countries of northern hemisphere. Although the etiology of UC remains unknown, cyclooxygenase-2 (COX-2)-dependent arachidonic acid (ARA) pathway of prostaglandins (PGs) biosynthesis is central to the pathology of UC. While increased PGE_2 levels are seen in active UC patients, those in remission have higher levels of an anti-inflammatory PG, PGD_2, signifying its role in resolution of inflammation. Therefore, strategies that can resolve inflammation by reducing PGE_2 levels can be potential therapies for UC. We have previously reported the redox regulatory ability of selenoproteins (SePr) to skew inflammatory pathways by modulation of ARA metabolism leading to decreased PGE_2 levels. In the current study, we found that SePr activated 15-hydroxy prostaglandin dehydrogenase (15PGDH)-dependent metabolic inactivation of PGE_2. Using a dextran sodium sulfate (DSS) model of experimental colitis, we demonstrate that Se supplementation at supraphysiological levels of 0.4 ppm and 1.0 ppm completely relieved colitis-associated inflammation. A concomitant increase in the expression and activity of 15-PGDH in the colon of these mice suggested that SePr were important in the regulation of PGE_2 metabolism leading to enhanced resolution. Studies in monocyte/macrophage-specific SePr knockout ($Trsp^{fl/fl}Cre^{LysM}$) mice suggested that SePr expression in these cells was pivotal for the resolution of colonic inflammation via the upregulation of 15PGDH expression. Thus, understanding the role of Se in the resolution of inflammation could be exploited to manipulate and regulate redox-dependent metabolism of key lipid metabolites as an adjunct therapy for colitis. Funded in part by US National Institutes of Health PHS grant DK077152 to KSP.

REDOX SELENIUM CHEMISTRY: TARGETING VIRUSES, BACTERIA AND CANCER (1988–2013)

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Purpose: To review the requirements for selenium (Se) redox cycling chemistry (1988) and demonstrate how to target Se to viruses, bacteria, and cancers for therapeutic applications (2013).

Background: Selenium compounds have been reported to be toxic perhaps as early as 1265 (Marco Polo). These toxic variations are inherent within the Se compounds ability to form selenide anions (RSe-), isoselenocyanates (RNCSe) and the rest of the compounds organic configuration. Selenite and selenate are not toxic per se. Organic selenide anions and isoselenocyantes that are toxic, are toxic because they redox cycle, forming superoxide (O_2^-) and other ROS. Because the Se compounds that are toxic are generally systemically toxic, affecting manly liver, spleen and keratin tissues, targeting Se toxicity using small molecules, existing drugs, vitamers, peptides and monoclonal antibodies is likely to avoid systemic toxicity while producing specific therapeutic toxicity.

Methodology: Many diselenides (RSeSeR) are reduced and form monoselenides (RSe-). Hydrophobic di- and monoselenides are useful to prevent biofilm formation on biopolymers. Halogens can be replaced with Se using KSeCN forming reducible selenocyanates (RSeCN). Selenocyanoproprionic acid, amines and alcohols can be attached to drugs and small molecules via standard organic chemical reactions. To primary amines of peptides, proteins and antibodies, selenides and isoselenocyanates can be covalently attached by modifying the Bolton-Hunter reagent.

Conclusions: Many Se compounds are toxic; redox cycle by oxidizing thiols and organic chemistry provides methods to target Se toxicity. The only requirements for Se pharmaceuticals are specific receptors, and or significant differentials between what is normal and the pathogenic viruses, bacteria or cancer cells. While other trace elements are also toxic, selenium to date stands alone owing to its organic attachment capabilities and better catalytic redox cycling activity.