Independent Studies

1. RibCys – High LET Radiation (NTRS)

Lenarczyk, M.; Ueno, A.; Vannais, D.B.; Kraemer, S.; Kronenberg, A.; Roberts, J.C.; Tatsumi, K.; Hei, T.K.; Waldron, C.A. The "Pro-drug" RibCys Decreases the Mutagenicity of High-LET Radiation in Cultured Mammalian Cells. Radiation Research, 2003, 160, 579-583.

Abstract

We are carrying out studies aimed at reducing the mutagenic effects of high-LET 56Fe ions and 12C ions (56Fe ions, 143 keV/microm; 12C ions, 100 keV/microm) with certain drugs, including RibCys [2-(R,S)-D-ribo-(1',2',3',4'-tetrahydroxybutyl)-thiazolidine-4(R)-carboxylic acid]. RibCys, formed by condensation of L-cysteine with D-ribose, is designed so that the sulfhydryl amino acid L-cysteine is released intracellularly through nonenzymatic ring opening and hydrolysis leading to increased levels of glutathione (GSH). RibCys (4 or 10 mM), which was present during irradiation and for a few hours after, significantly decreased the yield of CD59- mutants induced by radiation in AL human-hamster hybrid cells. RibCys did not affect the clonogenic survival of irradiated cells, nor was it mutagenic itself. These results, together with the minimal side effects reported in mice and pigs, indicate that RibCys may be useful, perhaps even when used prophylactically, in reducing the mutation load created by high-LET radiation in astronauts or other exposed individuals.

2. Effect of Ribose Cysteine Pretreatment

Lucas Slitt, A.M.; Dominick, P.K.; Roberts, J.C.; Cohen, S.D. Effect of Ribose Cysteine Pretreatment on Hepatic and Renal Acetaminophen Metabolite Formation and Glutathione Depletion. Basic Clin. Pharmacol. Toxicol., 2005, 96 (6), 487-94.

Results

- Ribose cysteine treatment reversed the acetaminophen-induced decline in hepatic non-protein sulfhydryl and renal GSH.
- Ribose cysteine treatment altered acetaminophen metabolite concentration in liver and kidney.
- Ribose cysteine does not inhibit acetaminophen activation in vitro.
- Since Buthionine sulfoximine pretreatment prevented the de novo biosynthesis of glutathione from RibCys, the mechanism by which RibCys protected against acetaminophen was by the synthesis of new glutathione.

*Acetaminophen = Tylenol

3. GSH and Disease Progression

Ballatori N, Krance SM, Notenboom S, Shi S, Tieu K, Hammond CL. Glutathione dysregulation and the etiology and progression of human diseases. Biol Chem., 2009 Mar; 390(3):191-214.

Abstract

Glutathione (GSH) plays an important role in a multitude of cellular processes, including cell differentiation, proliferation, and apoptosis, and as a result, disturbances in GSH homeostasis are implicated in the etiology and/or progression of a number of human diseases, including cancer, diseases of aging, cystic fibrosis, and cardiovascular, inflammatory, immune, metabolic, and neurodegenerative diseases. Owing to the pleiotropic effects of GSH on cell functions, it has been quite difficult to define the role of GSH in the onset and/or the expression of human diseases, although significant progress is being made. GSH levels, turnover rates, and/or oxidation state can be compromised by inherited or acquired defects in the enzymes, transporters, signaling molecules, or transcription factors that are involved in its homeostasis, or from exposure to reactive chemicals or metabolic intermediates. GSH deficiency or a decrease in the GSH/glutathione disulfide ratio manifests itself largely through an increased susceptibility to oxidative stress, and the resulting damage is thought to be involved in diseases, such as cancer, Parkinson's disease, and Alzheimer's disease. In addition, imbalances in GSH levels affect immune system function, and are thought to play a role in the aging process. Just as low intracellular GSH levels decrease cellular antioxidant capacity, elevated GSH levels generally increase antioxidant capacity and resistance to oxidative stress, and this is observed in many cancer cells. The higher GSH levels in some tumor cells are also typically associated with higher levels of GSH-related enzymes and transporters. Although neither the mechanism nor the implications of these changes are well defined, the high GSH content makes cancer cells chemoresistant, which is a major factor that limits drug treatment. The present report highlights and integrates the growing connections between imbalances in GSH homeostasis and a multitude of human diseases.

4. Heavy Metals and Human Health

Jan AT, Azam M, Siddiqui K, Ali A, Choi I, Haq QM. Heavy Metals and Human Health: Mechanistic Insight into Toxicity and Counter Defense System of Antioxidants. Int J Mol Sci., 2015 Dec 10; 16(12):29592-630.

Abstract

Heavy metals, which have widespread environmental distribution and originate from natural and anthropogenic sources, are common environmental pollutants. In recent decades, their contamination has increased dramatically because of continuous discharge in sewage and untreated industrial effluents. Because they

are non-degradable, they persist in the environment; accordingly, they have received a great deal of attention owing to their potential health and environmental risks. Although the toxic effects of metals depend on the forms and routes of exposure, interruptions of intracellular homeostasis include damage to lipids, proteins, enzymes, and DNA via the production of free radicals. Following exposure to heavy metals, their metabolism and subsequent excretion from the body depends on the presence of antioxidants (glutathione, α-tocopherol, ascorbate, etc.) associated with the quenching of free radicals by suspending the activity of enzymes (catalase, peroxidase, and superoxide dismutase). Therefore, this review was written to provide a deep understanding of the mechanisms involved in eliciting their toxicity in order to highlight the necessity for development of strategies to decrease exposure to these metals, as well as to identify substances that contribute significantly to overcome their hazardous effects within the body of living organisms.

Conclusions

Agents responsible for multiple human complications vary grossly in their physiochemical properties, and metals are no exception. After entering an ecosystem, metals induce a broad range of physiological, biochemical, and behavioural dysfunctions via induction of oxidative stress in humans. Oxidative and nitrative stress developed in response to toxicants plays an important role in damaging biomolecules, as well as disrupting signalling pathways, which in turn leads to pathogenesis of multiple human diseases. Despite the protection afforded by the cellular redox environment in biological systems, its disruption due to exogenous stimuli or endogenous metabolic alteration leads to increased intracellular ROS/RNS levels. Buffering and muffling reactions between ROS/RNS generation and elimination to redress the deleterious effects caused by oxidative stress are maintained by complex antioxidant (enzymatic and non-enzymatic) systems. In terms of a reactivity standpoint, the enzymatic antioxidant system constitutes the first line of defence, followed by reduced thiols and low molecular weight antioxidants and then by a broad range of products from dietary sources. Defense systems for overcoming the deleterious effects of oxidative and nitrative stress generated by production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) are essential to maintenance of cellular homeostasis. However, depletion of the cellular antioxidant pool characterized by (a) increased ROS and RNS production; (b) depletion of free-radical scavengers (Vitamins E and C) and cellular antioxidants (largely GSH); and (c) inhibition of the activity of enzymes such as (GPx) glutathione peroxidase, GSH-reductase, GSH-transferase, catalase (CAT) and superoxide dismutase (SOD) that contribute significantly to the metabolism and detoxification of reactive oxygen species (ROS). Having grave consequences within the bodies of living organisms, maintaining the availability of essential and controlled distribution of toxic metal ions is an efficient means of protection against the deleterious effects of heavy metals. Accordingly, an improved understanding of the counter-productive and beneficial defensive mechanisms of dietary antioxidants support their role in therapeutics for metal induced oxidative stress. To bridge this knowledge gap, studies pertaining to elucidation of molecular mechanisms involved in imparting

toxicity imposed by heavy metals are essential. In addition, detailed mechanistic studies underlying the beneficial effects of dietary antioxidants for their optimum dosage and duration of treatment will be helpful in the development of combinatorial strategies as part of effective treatment regimes for better clinical recovery in metal intoxication cases.

5. Free Radicals, Oxidative Stress, Antioxidants, Glutathione

Lobo V, Patil A, Phatak A, and Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev., 2010 Jul-Dec; 4(8): 118–126.

Abstract

In recent years, there has been a great deal of attention toward the field of free radical chemistry. Free radicals reactive oxygen species and reactive nitrogen species are generated by our body by various endogenous systems, exposure to different physiochemical conditions or pathological states. A balance between free radicals and antioxidants is necessary for proper physiological function. If free radicals overwhelm the body's ability to regulate them, a condition known as oxidative stress ensues. Free radicals thus adversely alter lipids, proteins, and DNA and trigger a number of human diseases. Hence application of external source of antioxidants can assist in coping this oxidative stress. Synthetic antioxidants such as butylated hydroxytoluene and butylated hydroxyanisole have recently been reported to be dangerous for human health. Thus, the search for effective, nontoxic natural compounds with antioxidative activity has been intensified in recent years. The present review provides a brief overview on oxidative stress mediated cellular damages and role of dietary antioxidants as functional foods in the management of human diseases.

Keywords: Ageing; antioxidant; free radicals; oxidative stress.

Some internally generated sources of free radicals are [8]:

- Mitochondria
- Xanthine oxidase
- Peroxisomes
- Inflammation
- Phagocytosis
- Arachidonate pathways
- Exercise
- Ischemia/reperfusion injury

Some externally generated sources of free radicals are:

- Cigarette smoke
- Environmental pollutants
- Radiation

- Certain drugs, pesticides
- Industrial solvents
- Ozone

[8] Ebadi M. Antioxidants and free radicals in health and disease: An introduction to reactive oxygen species, oxidative injury, neuronal cell death and therapy in neurodegenerative diseases. *Arizona:* Prominent Press; 2001.

CONCEPT OF OXIDATIVE STRESS

The term is used to describe the condition of oxidative damage resulting when the critical balance between free radical generation and antioxidant defenses is unfavorable.[14] Oxidative stress, arising as a result of an imbalance between free radical production and antioxidant defenses, is associated with damage to a wide range of molecular species including lipids, proteins, and nucleic acids.[15] Short-term oxidative stress may occur in tissues injured by trauma, infection, heat injury, hypertoxia, toxins, and excessive exercise. These injured tissues produce increased radical generating enzymes (e.g., xanthine oxidase, lipogenase, cyclooxygenase) activation of phagocytes, release of free iron, copper ions, or a disruption of the electron transport chains of oxidative phosphorylation, producing excess ROS. The initiation, promotion, and progression of cancer, as well as the side-effects of radiation and chemotherapy, have been linked to the imbalance between ROS and the antioxidant defense system. ROS have been implicated in the induction and complications of diabetes mellitus, age-related eye disease, and neurodegenerative diseases such as Parkinson's disease.[16]

- Oxidative stress and human diseases
- Cardiovascular diseases
- Carcinogenesis
- Free radical and aging
- Oxidative damage to protein
- Lipid peroxidation
- Oxidative damage to DNA
- ANTIOXIDANTS
- Glutathione

[14] Rock CL, Jacob RA, Bowen PE. Update on the biological characteristics of the antioxidant micronutrients: vitamin C, vitamin E, and the carotenoids. J Am Diet Assoc. 1996;96:693–702. [PubMed]
[15] Mc Cord JM. The evolution of free radicals and oxidative stress. Am J Med. 2000;108:652–9. [PubMed]
[16] Rao AL, Bharani M, Pallavi V. Role of antioxidants and free radicals in health and disease. Adv Pharmacol Toxicol. 2006;7:29–38.

Conclusion

Free radicals damage contributes to the etiology of many chronic health problems such as cardiovascular and inflammatory disease, cataract, and cancer. Antioxidants prevent free radical induced tissue damage by preventing the formation of radicals, scavenging them, or by promoting their decomposition. Synthetic antioxidants are recently reported to be dangerous to human health.

Thus, the search for effective, nontoxic natural compounds with antioxidative activity has been intensified in recent years. In addition to endogenous antioxidant defense systems, consumption of dietary and plant-derived antioxidants appears to be a suitable alternative. Dietary and other components of plants form a major source of antioxidants. The traditional Indian diet, spices, and medicinal plants are rich sources of natural antioxidants; higher intake of foods with functional attributes including high level of antioxidants in antioxidants in functional foods is one strategy that is gaining importance.

Newer approaches utilizing collaborative research and modern technology in combination with established traditional health principles will yield dividends in near future in improving health, especially among people who do not have access to the use of costlier western systems of medicine.

6. Glutathione S-transferase T1-1 (GSTT1-1) Is Lacking in 80% of Asians

Landi S. Mammalian class theta GST and differential susceptibility to carcinogens: a review. *Mutat. Res*., 2000; 463:247–83.

Abstract

Glutathione S-transferases (GSTs) are an important part of the cellular detoxification system and, perhaps, evolved to protect cells against reactive oxygen metabolites. Theta is considered the most ancient among the GSTs and theta-like GSTs are found in mammals, fish, insects, plants, unicellular algae, and bacteria. It is thought that an ancestral theta-gene underwent an early duplication before the divergence of fungi and animals and further duplications generated the variety of the other classes of GSTs (alpha, mu, phi, etc.). The comparison of the aminoacidic homologies among mammals suggests that a duplication of an ancient GST theta occurred before the speciation of mammals and resulted in the subunits GSTT1 and GSTT2. The ancestral GST theta has a dehalogenase activity towards several halogenated compounds, such as the dichloromethane. In fact, some aerobic and anaerobic methylotrophic bacteria can use these molecules as the sole carbon and energy source. The mammalian GST theta cannot sustain the growth of bacteria but still retains the dehalogenating activity. Therefore, although mammalian GST theta behaves as a scavenger towards electrophiles, such as epoxides, it acts also as metabolic activator for halogenated compounds, producing a variety of intermediates potentially dangerous for DNA and cells. For example, mice exposed to dichloromethane show a dose-dependent incidence 39 of cancer via the GSTT1-1 pathway. Because GSTT1-1 is polymorphic in humans, with about 20% of Caucasians and 80% of Asians lacking the enzyme, the relationship between the phenotype and the incidence of cancer has been investigated extensively in order to detect GSTT1-1-associated differential susceptibility towards endogenous or exogenous carcinogens. The lack of the enzyme is related to a slightly increased risk of cancer of the bladder, gastro-intestinal tract, and for tobacco-related tumors (lung or oral cavity). More pronounced risks were found

in males with the GSTT1-null genotype for brain diseases and skin basal cell carcinomas not related to sunlight exposures. Moreover, there was an increased risk of kidney and liver tumors in humans with the GSTT1-1 positive genotype following exposures to halogenated solvents. Interestingly, the liver and kidney are two organs that express the highest level of GST theta in the human body. Thus, the GSTT1-1 genotype is suspected to confer decreased or increased risk of cancer in relation to the source of exposure; in vitro studies, mostly conducted on metabolites of butadiene, confirm the protective action of GSTT1-1, whereas, thus far, experimental studies prove that the increasing risk is limited.