

INDEPENDENT STUDIES

RiboCeine™ – GSH – Antioxidants

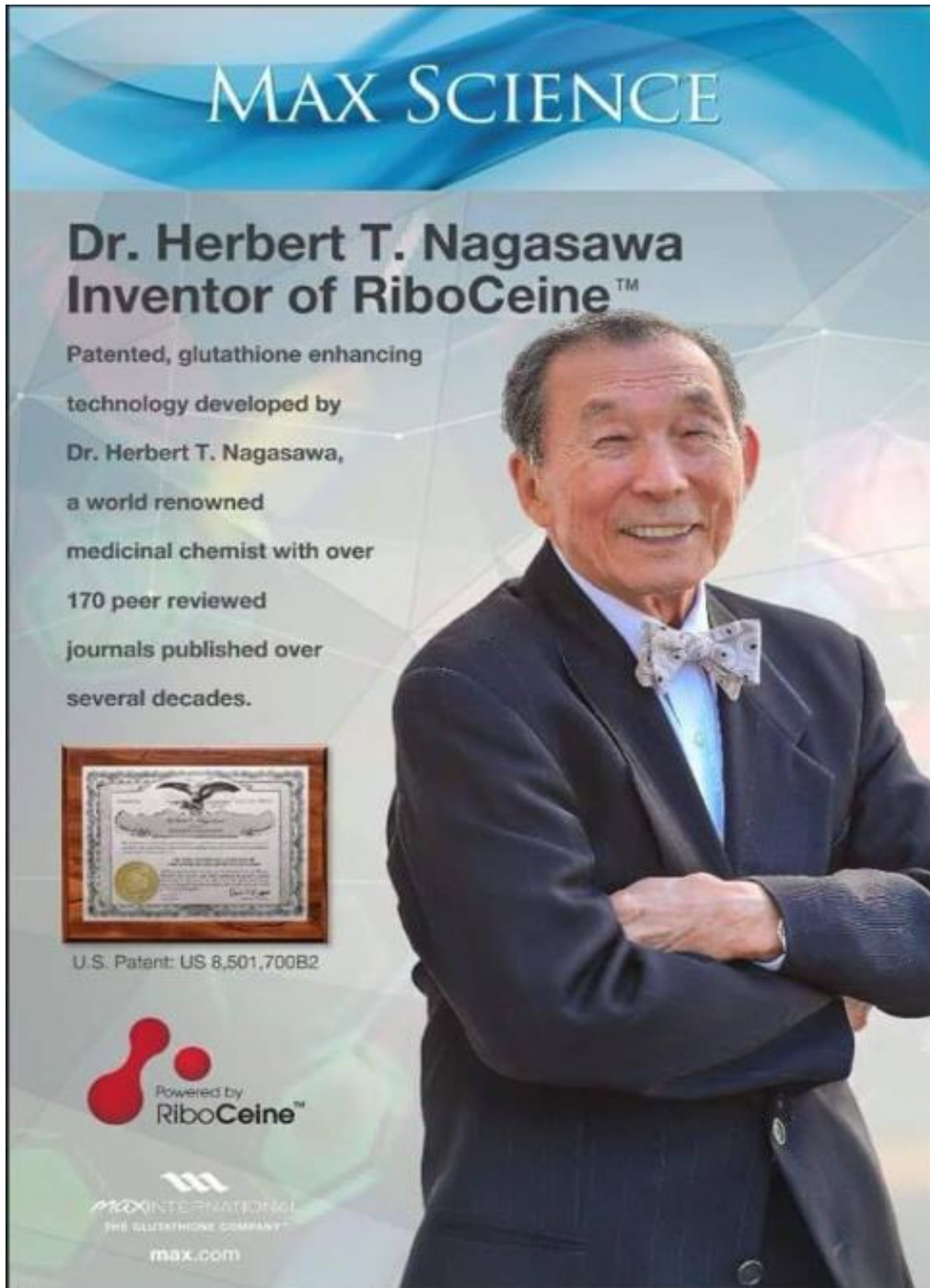


Compiled by Jennifer Tsai

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MAX SCIENCE



MAX SCIENCE

Dr. Herbert T. Nagasawa
Inventor of RiboCeine™

Patented, glutathione enhancing
technology developed by
Dr. Herbert T. Nagasawa,
a world renowned
medicinal chemist with over
170 peer reviewed
journals published over
several decades.

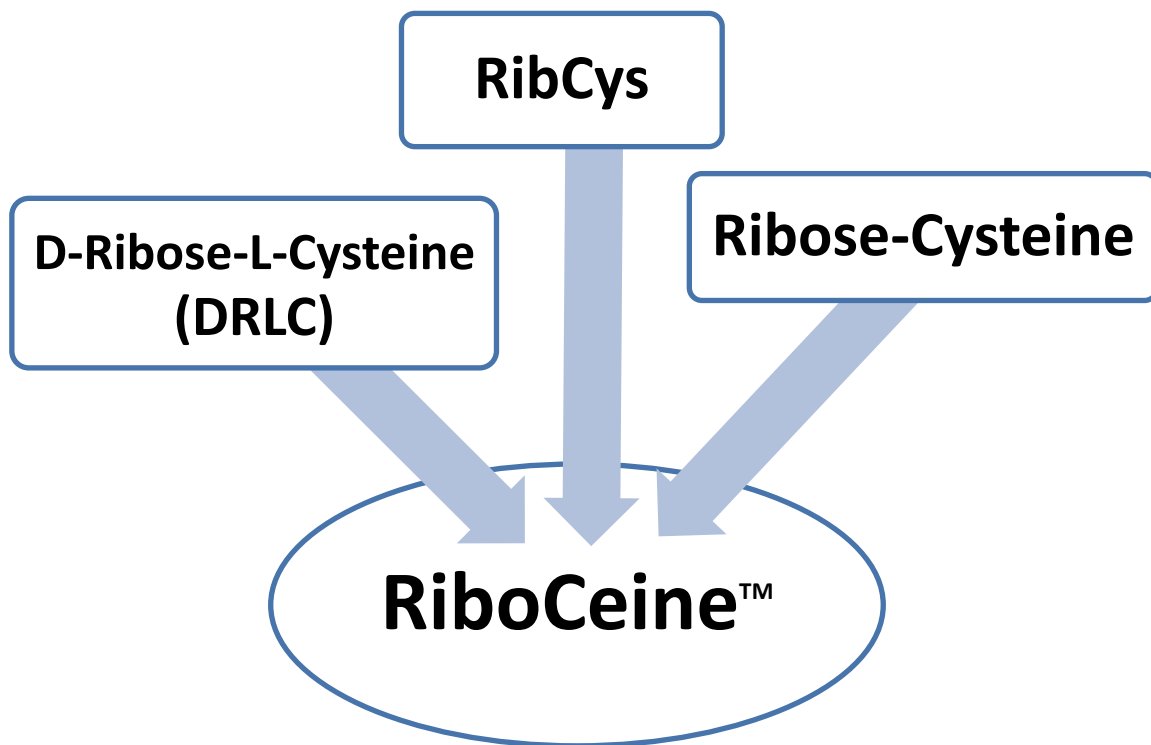


U.S. Patent: US 8,501,700B2

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RIBOCEINE™



RiboCeine = RibCys = Ribose-Cysteine = DRLC

RIBCYS PATENT BY MAX



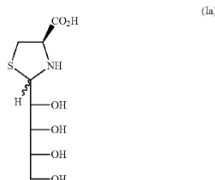
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 Nagasawa (43) **Pub. Date: Nov. 28, 2013**

(54) METHOD TO ENHANCE DELIVERY OF GLUTATHIONE AND ATP LEVELS IN CELLS (71) Applicant: Max International LLC, Salt Lake City, UT (US) (72) Inventor: Herbert T. Nagasawa, Irvine, CA (US) (73) Assignee: Max International LLC, Salt Lake City, UT (US) (21) Appl. No.: 13/958,530 (22) Filed: Aug. 2, 2013 Related U.S. Application Data (63) Continuation of application No. 12/182,354, filed on Jul. 30, 2008, now Pat. No. 8,501,700, which is a	continuation of application No. 10/990,933, filed on Nov. 17, 2004, now abandoned. Publication Classification (51) Int. Cl. <i>A61K 31/426</i> (2006.01) (52) U.S. Cl. CPC <i>A61K 31/426</i> (2013.01) USPC 514/365 (57) ABSTRACT A therapeutic method is provided comprising treating a mammal subject to hypoxia with an amount of 2(R,S)-D-ribo-(1', 2', 3', 4'-tetrahydroxybutyl)thiazolidine-4(R)-carboxylic acid (RibCys) or a pharmaceutically acceptable salt thereof effective to both maintain, restore or increase both the ATP levels and the glutathione (GSH) levels in said tissue.
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SUMMARY OF THE INVENTION

[0015] The present invention provides a method to treat a mammal threatened by, or afflicted with a hypoxic condition (hypoxia) comprising administering an effective amount of a compound of formula (Ia):

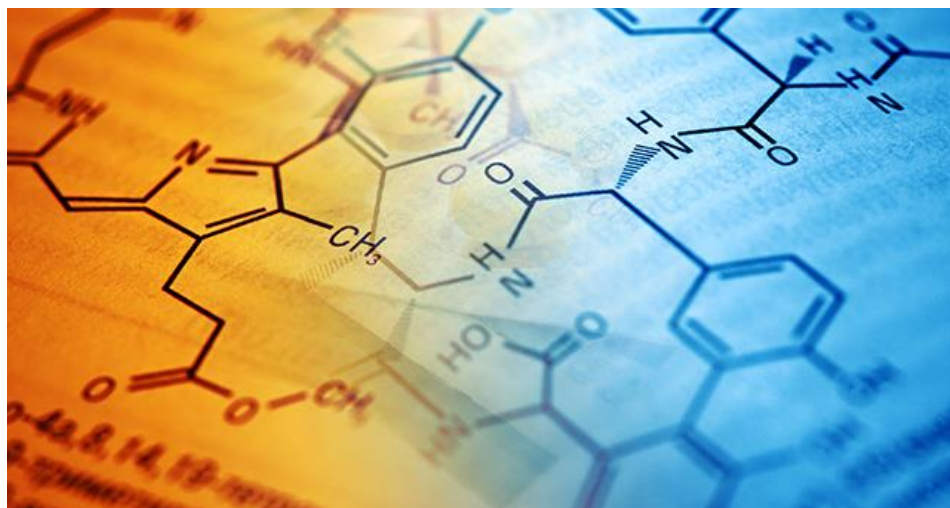


(RibCys) or a pharmaceutically acceptable salt thereof, effective to counteract the effects of said hypoxia in the tissue(s) of said mammal. Although depressed glutathione levels have been implicated in a number of hypoxic conditions, as discussed above, the use of RibCys or its salts to prevent, counteract or otherwise treat such conditions has

not been reported. It is believed that simply administering a GSH precursor such as cysteine will not be as effective in many instances of hypoxia, when the depletion of ATP stores contributes to inhibition to the biosynthesis of GSH. As well as functioning as a prodrug for cysteine, administration of effective amounts of RibCys can deliver amounts of ribose to ATP-depleted tissues that stimulate the in vivo synthesis of ATP and that also can stimulate the synthesis of NADPH (nicotinamide adenine dinucleotide phosphate, reduced). This coenzyme supplies the electrons to glutathione reductase, which in turn recycles oxidized GSH via GSSG, to free GSH, which resumes its protective role as a cofactor for antioxidant enzymes in the cell. Optionally, compound (Ia) can be administered With an additional amount of free ribose. Preferably, administration will be by oral administration, particularly in prophylactic or pre-loading situations, but parenteral administration, as by injection or infusion, may be necessary in some situations.



INDEPENDENT STUDIES ON RIBOCEINE



Independent Studies on Ribocaine

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- 22.** Roberts, J.C.; Nagasawa, H.T.; Zera, R.T.; Fricke, R.F.; Goon, D.J. W. [Prodrugs of L-cysteine as protective agents against acetaminophen-induced hepatotoxicity. 2-\(polyhydroxyalky\)-and 2-\(Polyacetoxalky\)-Thiazolidine-4\(R\)-Carboxylic Acids. .](#) *Med Chem.*, **1987**, 30, 1891-1896.



SUMMARY OF KEY RIBOCEINE STUDIES ADAPTED FROM ORIGINAL PUBLICATIONS

By Scott Nagasawa, Pharm.D.

Max R&D, LLC – April 7, 2016



1. RiboCeine Shown to Protect Against Oxidative Stress

Roberts JC, Nagasawa HT, Zera RT, Fricke RF, Goon DJW. Prodrugs of L-Cysteine as Protective Agents against Acetaminophen-Induced Hepatotoxicity. 2-(Polyhydroxyalkyl)- and 2-(Polyacetoxyalkyl) thiazolidine-4(R)-carboxylic Acids. *Journal of Medicinal Chemistry.* 1987, 30:1891-1896.

Study Background

Oxidative stress is a metabolic condition where excess free radicals are being produced. Chronic oxidative stress has been associated with many diseases and disorders including cardiovascular diseases, neurodegenerative disorders, cataract formation, inflammatory diseases, etc. Although there is no proven relationship that oxidative stress is the cause of these diseases, there is a significant body of evidence that oxidative stress (low glutathione levels) is prevalent in many disease states and disorders.

Basic science researchers in pursuit of determining whether certain compounds are effective in reducing oxidative stress require animal models to conduct their experiments. To mimic chronic oxidative stress in animals, an acute oxidative stress model is used to permit these experiments to be conducted in shorter time periods, thereby saving on time and cost. A widely accepted acute oxidative stress animal model is the acetaminophen over-dose model, wherein severe depletion of liver glutathione is manifested when toxic doses are administered.

Acetaminophen, a widely used pain killer, is not toxic at recommended doses, and is readily metabolized by the liver to non-toxic products, which are then excreted in the urine. However at high or toxic doses, the normal metabolic pathways become overwhelmed and the liver will, ironically, metabolize acetaminophen through a different pathway that produces a very reactive and toxic metabolite, thus triggering the development of acute oxidative stress. This toxic metabolite is detoxified by liver glutathione; however, once liver glutathione becomes depleted, this toxic metabolite will react with liver cells leading to liver necrosis and eventually death of the animals.

To overcome acetaminophen-induced acute oxidative stress that causes severe depletion of liver glutathione, researchers administer test compounds believed to promote varying degrees of glutathione production. The compound's effectiveness is determined by the level of protection it provides to the liver, and its ability to prevent animal death. Those compounds that exhibit the best protection to the liver and having the highest animal survival rates are deemed to be the superior compounds. The level of protection directly correlates with the compound's ability to improve liver glutathione levels.

Summary: In this study, a total of 8 sulfhydryl-protected L-cysteine compounds, in addition to N-acetylcysteine (NAC) which is *not* sulfhydryl-protected, were evaluated in an *in vivo* mouse model to determine their ability to protect against liver toxicity from a high dose of acetaminophen. RiboCeine was among the sulfhydryl-protected compounds tested. In protecting the sulfhydryl (SH) group of L-cysteine, the authors used naturally-occurring endogenous substances to avoid any possibility of toxicity by the protecting substance itself.

Part 1: The experimental protocol involved the administration of a lethal dose (LD₉₀) of acetaminophen where 90% of the mice were expected not to survive plus the test compounds. Toxicity was assessed on the basis of overall survival of the animals at 48 hours, as well as histological (cell pathology) criteria of liver cell damage assessed by an independent third party "who had no knowledge of the experimental protocols or sample identity". RiboCeine was the only compound tested where no animal death occurred at 48 hours. All other compounds tested had varying rates of animal survival between 30% to 94%. The animals without intervention had only a 17% survival rate. RiboCeine "showed the best histological profile" with all animals showing a necrosis rating of +2 or below (range 4+ to 0). All other compounds tested had animals with histological ratings of 4+.

Part 2: In the *in vitro* study with cultured hepatocytes (liver cells), RiboCeine at 40% of the dose of NAC raised liver cell glutathione levels 130% better than NAC. Since BSO, an inhibitor of glutathione synthesis, prevented this glutathione increase, it was concluded that the L-cysteine from RiboCeine must have been utilized in the newly formed glutathione.

Conclusion: The collective *in vivo* and *in vitro* data suggested that RiboCeine was readily bioavailable, was superior to the other compounds tested, and should serve as an effective cysteine delivery system to the liver.

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RIBOCEINE WAS SHOWN TO BE 300% MORE EFFECTIVE THAN NAC IN RAISING LIVER GLUTATHIONE LEVELS

Journal of Medicinal Chemistry, 1987, Vol. 30, No. 10 1893

Table I. Increased GSH Content of Rat Hepatocytes after Incubation with L-Cysteine Prodrugs

prodrug	concn, mM	[GSH] $\pm$ SE, nmol/10 ⁶ cells	[GSH] rel to controls
none (control)		35.4 $\pm$ 0.78	1.0
GlcCys (1g)	1.0	75.2 $\pm$ 2.15	2.1
RibCys (1d)	1.0	61.2 $\pm$ 1.52	1.7
XylCys (1e)	1.0	58.3 $\pm$ 0.99	1.6
GalCys (1f)	1.0	58.0 $\pm$ 2.00	1.6
ManCys (1h)	1.0	57.8 $\pm$ 0.87	1.6
GlycCys (1a)	1.0	46.1 $\pm$ 1.10	1.3
LyxCys (1c)	1.0	45.9 $\pm$ 1.95	1.3
AraCys (1b)	1.0	42.9 $\pm$ 2.17	1.2
<i>N</i> -acetyl-L-cysteine (NAC)	2.5	45.8 $\pm$ 1.27	1.3

On the table above, even though 2 ½ times more NAC was used in the incubation of these hepatocytes, the glutathione levels were 30% lower than the cells that were incubated with RiboCeine (RibCys). Therefore, RiboCeine was at least 300% more effective in raising liver cell glutathione than NAC was in this liver cell model.

2. Organ Glutathione Levels after RiboCeine

Roberts, J.C.; Francetic, D.J. Time course for the elevation of glutathione in numerous organs of L1210-bearing CDF1 mice given the L-cysteine prodrug, RibCys. *Toxicology Letters*, 1991, 59, 245-251.

Study Background

It is well accepted by the scientific community that glutathione levels in cells are homeostatically controlled, and dramatic elevations in glutathione levels are not expected because its biosynthesis is stringently controlled by feedback inhibition¹. Therefore, glutathione may only achieve a certain level before the biosynthetic machinery would be turned off, regardless of the level of amino acid precursors available in the cell¹. However, in the presence of a toxic substance that requires glutathione for detoxification, the levels of glutathione in the liver are continually decreasing and virtually depleted in some cases (acetaminophen overdoses). The value of these glutathione elevation studies is not in the “absolute increase of glutathione achieved”, but rather the compound’s ability to maintain glutathione levels under oxidative stress conditions.

Glutathione levels in many organs fall as the result of fasting, and therefore, compounds can be tested in fasting animals for their ability to elevate and maintain organ glutathione.

Summary: In this study, RibCys (RiboCeine) was evaluated in fasting mice for its ability to improve glutathione levels in numerous organs at different time points after RiboCeine administration. After 8 to 10 hours of fasting, glutathione levels dropped approximately 43% in the liver, 41% in the bladder, 31% in the kidney, 25% in the heart and 60% in muscle. Other organs, such as the spleen, pancreas and lung, showed no significant differences in glutathione levels between the two nutritional states.

Results: “Glutathione in the liver was elevated 1.5 fold compared to untreated controls at the 16 hour time point. Kidney glutathione also was maximal at 16 hours and achieved 1.6 times control values. Glutathione in muscle achieved 2.5 times the levels in control animals, while the bladder was elevated 2.1 fold and the heart 1.8 fold.”

Conclusion: The authors concluded that “RibCys was able to maintain, and, in some organs, continued to elevate glutathione even though the animals were subject to continued fasting”. “The results from the present studies support the hypothesis that RibCys can serve as a reservoir for the crucial glutathione precursor, L-cysteine, and continually supply the amino acid as glutathione synthesis proceeds.”

¹ Meister A, J., Glutathione metabolism and its selective modification. *Biol. Chem.* 1988, 273, 17205-17208.

3. Inflammatory Bowel Disease and Glutathione

Oz HS, Chen TS, Nagasawa H. Comparative efficacies of two cysteine prodrugs and a glutathione delivery agent in a colitis model. *Translational Research*, 2007:150(2), 122-129.

Study Background

Glutathione is the most important endogenous antioxidant for cellular defense against oxidative stress, and is vital for the integrity of the gut. Oxidant-mediated injury plays an important role in the pathophysiology of inflammatory bowel disease (IBD).

Reactive oxygen species (ROS) have been implicated in the tissue destruction observed in IBD. These ROS include hydroxyl radical, superoxide radical, hydrogen peroxide and nitric oxide. ROS are extremely unstable because of their high reactivity, and can lead to lipid peroxidation and the oxidation of DNA and proteins.

Summary: Dextran sodium sulfate (DSS) induced colitis is a well-accepted model that produces colonic inflammation of the gut. This study compared the protective effects of three glutathione promoting agents that included RiboCeine. In this mouse model of colitis, these compounds were incorporated into the daily chow. The animals were provided with normal drinking water, but after three days, the water was supplemented with DSS. All animals remained on the assigned diets until they were euthanized on day 10. Mice administered DSS developed severe colitis and suffered weight loss.

Results: Colonic lesions significantly improved when RiboCeine was incorporated in the food ($P < 0.001$). Colon lengths of the colitis mice (DSS) were significantly decreased because of mucosal inflammation, edema and thickening (control 109 ± 2.8 mm versus DSS 66 ± 2.8 mm, $P < 0.001$). In contrast, this decrease in colon length was significantly attenuated when treated with RiboCeine (84 ± 4.2 mm, $P < 0.001$) which is consistent with reduced inflammation of their colonic tissue. The mice receiving RiboCeine/chow had improved weight gains with no significant difference noted

between normal control mice (control 29 ± 0.9 ; DSS 26 ± 1.0 grams) and RiboCeine (29 ± 0.9 grams). Liver concentration of reduced glutathione, the major source of gut antioxidant, was depleted in colitis animals (DSS $4.81 \pm$ -micromole/gram; control $6.53 \pm$ -micromole/gram) and normalized with RiboCeine administration ($P > 0.05$).

Conclusion: RiboCeine reduced disease activity in the mouse model of DSS induced colitis by restoring colonic glutathione, and may be a useful dietary supplement for the prevention or possible palliation of IBD in humans.

<http://www.sciencedirect.com/science/article/pii/S0194599810006455?np=y>

<http://www.ncbi.nlm.nih.gov/pubmed/20723783>

4. Antioxidant Therapy Prevents Age-Related Hearing Loss

Heman-Ackah SE, Juhn SK, Huang TC, Wiedmann TS. A combination antioxidant therapy prevents age-related hearing loss in C57BL/6 mice. *Otolaryngology-Head and Neck Surgery*. 2010:143,429-434.

Study Background

Age-related hearing loss (ARHL or presbycusis) refers to a gradual, progressive hearing loss that accompanies aging. The hearing loss is typically described as downward-sloping high frequency loss but may be associated with various types of system dysfunctions that progress with aging.

Age-related hearing loss is one of the most common conditions affecting the elderly population. Approximately 35 percent of adults age 65 and older have been reported to have some degree of age-related hearing loss. It is projected that by 2025, approximately 24.5 million Americans will be affected. Age-related hearing loss disables an individual's ability to communicate, thereby effectively jeopardizing their autonomy. Patients often experience associated depression and social withdrawal. Thus, age-related hearing loss presents a major public health concern.

Oxidative injury caused by free-radical damage is perhaps the most fundamental cause of age-related pathology in the biological aging of cells. Oxidative damage may be an important intrinsic factor in the pathogenesis of presbycusis. Increased concentrations of free radicals [(ROS) and reactive nitrogen species (RNS)] are implicated as a mediator of oxidative stress and damage to the inner ear in ARHL.

Summary: This study uses a strain of mice that progressively lose their hearing over their lifetime, and is a widely accepted model for the study of ARHL. A combination antioxidant cocktail which included RiboCeine, a critical precursor of cysteine for the biosynthesis of glutathione, was administered. The rationale for creating a

combination of antioxidant treatment was to target multiple sites within the oxidative pathway to retard or prevent oxidative stress.

Results: The data demonstrated that the administration of this combination of antioxidants to this animal model greatly attenuated the presentation of ARHL at all frequency levels compared to the control group.

Conclusion: The administration of combination antioxidants, which included RiboCeine, attenuated the presentation of AHRL, suggesting that such combinations might be useful in preventing or delaying ARHL, a major public health problem.

5. RiboCeine and Cardiovascular Health

Kader, T.; Porteous C.M.; Williams M.J.A.; Giese, S.P.; McCormick, S.P.A.

Ribose-cysteine increases glutathione-based antioxidant status and reduces LDL in human lipoprotein(a) mice. *Atherosclerosis*. 2014, 237, 725-733.

Overview: Glutathione is a co-factor for glutathione peroxidase, the enzyme that catalyzes the detoxification of lipid peroxides. A low glutathione peroxidase activity and increased oxidized lipids are associated with cardiovascular disease.¹

Methods: Human lipoprotein(a) transgenic mice were treated with 4 mg/day ribose-cysteine (0.16 g/kg body weight) for 8 weeks. Livers and blood were harvested from treated and untreated controls (n = 9 per group) and glutathione concentrations, glutathione peroxidase activity, thiobarbituric acid reactive substances (TBARS), 8-isoprostanes and plasma lipid concentrations were measured.

Results: Ribose-cysteine increased glutathione concentrations in the liver and plasma ($P < 0.05$). Glutathione peroxidase activity was increased in both liver (1.7 fold, $P < 0.01$) and erythrocytes (3.5 fold, $P < 0.05$). TBARS concentrations in the liver, plasma and aortae were significantly reduced with ribose-cysteine ($P < 0.01$, $P < 0.0005$ and $P < 0.01$, respectively) as were the concentrations of 8-isoprostanes in the liver and aortae ($P < 0.0005$, $P < 0.01$, respectively). Ribose-cysteine treated mice showed significant decreases in Low Density Lipoproteins, human lipoprotein(a) and apoB concentrations ($P < 0.05$, $P < 0.01$ and $P < 0.05$, respectively), an effect which was associated with upregulation of the Low Density Lipoproteins receptor (LDLR).

Conclusion: As ribose-cysteine lowers Low Density Lipoproteins, human lipoprotein(a) and oxidized lipid concentrations, it might be an ideal intervention to increase protection against the development of atherosclerosis.

¹ Blankenberg, S. et al. Glutathione Peroxidase 1 Activity and Cardiovascular Events in Patients with Coronary Artery Disease. *New England Journal of Medicine*. 2003;349:1605-1613.

<http://www.americanjournalofsurgery.com/article/S0002-9610%2815%2900135-X/abstract>
<https://www.ncbi.nlm.nih.gov/pubmed/?term=25935230>

6. RiboCeine and Wound Healing

Saltman, A.E. D-Ribose-L-Cysteine supplementation enhances wound healing in a rodent model. *The American Journal of Surgery.* 2015;210:153-158.

Overview: Wound healing and chronic wounds are serious public health issues. While wounds heal, cellular stores of antioxidants are depleted. D-ribose-L-cysteine is a precursor to the antioxidant glutathione. The effect of oral supplementation with D-ribose-L-cysteine on wound healing was studied in rats.

Methods: A rodent model of calibrated wounding was used. Group A rats were given D-ribose-L-cysteine for 1 week before wounding and for 3, 7, or 14 days after wounding. Group B rats were given D-ribose-L-cysteine only after wounding. Control animals were given no supplement. Photographic comparisons were made to study wound edema and inflammation. Wound strength was determined by using a laser-vacuum device.

Results: During healing, both Group A and B animals showed less edema and inflammation than Control. Group A animals had the weakest wounds at 3 days after surgery, but the strongest wounds after 14 days. Group B animals had similar wound strength to Control animals at 7 days, but stronger wounds after 14 days.

Conclusion: D-ribose-L-cysteine supplementation appears to reduce wound inflammation early after wounding and enhance wound strength by 14 days. This suggests that increased intracellular glutathione levels may improve and enhance wound healing.



INDEPENDENT STUDIES



<http://ntrs.nasa.gov/search.jsp?R=20000109818#.T-uOyix66kl.mailto>

<https://www.ncbi.nlm.nih.gov/pubmed/?term=14565824>

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.

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The 'Pro-Drug' RibCys Decreases The Mutagenicity of High LET Radiation in Cultured Mammalian Cells

Author and Affiliation:
Lenarczyk, M. (Colorado State Univ., Dept. of Radiological Health Sciences, Fort Collins, CO United States);
Ueno, A. (Colorado State Univ., Dept. of Radiological Health Sciences, Fort Collins, CO United States);
Vannais, D. B. (Colorado State Univ., Dept. of Radiological Health Sciences, Fort Collins, CO United States);
Kraemer, S. (Colorado State Univ., Dept. of Radiological Health Sciences, Fort Collins, CO United States);
Kronenberg, A. (California Univ., Lawrence Berkeley Lab., Life Science Div., Berkeley, CA United States);
Roberts, J. C. (Utah Univ., Dept. of Medicinal Chemistry, Salt Lake City, UT United States);
Tatsumi, K. (National Inst. of Radiological Sciences, Div. of Biology and Oncology, Chiba, Japan);
Hei, T. K. (Columbia Univ., Center for Radiation Research, New York, NY United States);
Waldren, C. A. (Colorado State Univ., Dept. of Radiological Health Sciences, Fort Collins, CO United States)

Abstract:
We have initiated studies aimed at reducing the mutational effects of high LET radiation such as Fe-56 ions and C-12 ions with certain drugs. The mutagenicity of high LET (143 keV/micrometer) Fe-56 or C-12 ions (LET = 100 keV/micrometer) was quantified at the CD59 locus of human-hamster hybrid AL cells. RibCys [2,S)-D-ribo-(1',2',3',4'- Tetrahydroxybutyl)-thiazolidine-4(R)-ca riboxylic acid], formed by condensation of L-cysteine with D-ribose, is designed so that the sulfhydryl amino acid L-cysteine is released intracellularly via nonenzymatic ring opening and hydrolysis leading to increased levels of glutathione (GSH). RibCys (4 or 10 mM), present during irradiation and a few hours post-irradiation, significantly decreased the yield of CD59(-) mutants induced by radiation. 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 load of mutations created by high LET radiation in astronauts or other exposed individuals. RibCys is an attractive drug that may reduce the risk of carcinogenesis in people exposed to high LET radiation.

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http://onlinelibrary.wiley.com/doi/10.1111/j.1742-7843.2005.pto_96613.x/full

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.

Abstract ▼

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Basic Clin Pharmacol Toxicol. 2005 Jun;96(6):487-94.

Effect of ribose cysteine pretreatment on hepatic and renal acetaminophen metabolite formation and glutathione depletion.

Slitt AM¹, Dominick PK, Roberts JC, Cohen SD.

☰ Author information

¹Toxicology Program, Department of Pharmaceutical Sciences, University of Connecticut, Storrs, CT, USA.

Abstract

Ribose cysteine (2(R,S)-D-ribo-(1',2',3',4'-tetrahydroxybutyl)thiazolidine-4(R)-carboxylic acid) protects against acetaminophen-induced hepatic and renal toxicity. The mechanism for this protection is not known, but may involve inactivation of the toxic electrophile via enhancement of glutathione (GSH) biosynthesis. Therefore, the goal of this study was to determine if GSH biosynthesis was required for the ribose cysteine protection. Male CD-1 mice were injected with either acetaminophen or acetaminophen and ribose cysteine. The ribose cysteine cotreatment antagonized the acetaminophen-induced depletion of non-protein sulfhydryls in liver as well as GSH in kidney. Moreover, ribose cysteine cotreatment significantly increased the concentration of acetaminophen-cysteine, hepatic acetaminophen-mercapturate in liver and renal acetaminophen-GSH metabolites in kidney 4 hr after acetaminophen. To determine whether protection against acetaminophen-induced liver and kidney damage involved ribose cysteine dependent GSH biosynthesis, buthionine sulfoximine was used to selectively block gamma-glutamylcysteine synthetase (gamma-GCS). Plasma sorbitol dehydrogenase (SDH) activity and blood urea nitrogen from mice pretreated with buthionine sulfoximine and challenged with acetaminophen indicated that both liver and kidney injury had occurred. While co-treatment with ribose cysteine had previously protected against acetaminophen-induced liver and kidney injury, it did not diminish the acetaminophen-induced damage to either organ in the buthionine sulfoximine-treated mice. In conclusion, ribose cysteine serves as a cysteine prodrug that facilitates GSH biosynthesis and protects against acetaminophen-induced target organ toxicity.

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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.



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Glutathione dysregulation and the etiology and progression of human diseases

Nazzareno Ballatori^{*}, Suzanne M. Krance, Sylvia Notenboom, Shujie Shi, Kim Tieu, and Christine L. Hammond

Department of Environmental Medicine, University of Rochester School of Medicine, Rochester, NY 14642

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. Because of GSH's pleiotropic effects 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 (GSSG) 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.

Summary

Changes in GSH levels and/or oxidation state have now been reported in nearly all major human diseases. Although in many cases these changes likely occur as a result of the underlying disease progression, in other cases these changes are closely linked to the onset and/or development of the disease. The growing recognition that GSH is involved in critical cell signaling pathways that are regulated by *S*-glutathionylation and/or by the thiol redox status, and that GSH may function as a neurotransmitter or neuromodulator, provides considerable new insight into possible links between GSH and disease progression, and raises additional questions that can be addressed experimentally. In addition, the recognition that GSH is intimately involved in so many disease states has generated considerable interest in identifying therapies aimed at modulating GSH levels so as to modulate disease risk or progression. Although such therapies have significant hurdles to overcome, they offer significant promise for many human diseases.

<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4691126/> (Free PMC Article)

<http://www.mdpi.com/1422-0067/16/12/26183> (MDPI Open Access)

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.

Int J Mol Sci. 2015 Dec; 16(12): 29592–29630.

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Review

Heavy Metals and Human Health: Mechanistic Insight into Toxicity and Counter Defense System of Antioxidants

Arif Tasleem Jan ^{1,*†}, Mudsser Azam ^{2,†}, Kehkashan Siddiqui ², Arif Ali ², Inho Choi ^{1,*} and Qazi Mohd. Rizwanul Haq ²

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¹ School of Biotechnology, Yeungnam University, Gyeongsan 712-749, Korea

² Department of Biosciences, Jamia Millia Islamia, New Delhi 110025, India; azammudsser@gmail.com (M.A.); kehkashansiddiqui7@gmail.com (K.S.); ali.arifali@gmail.com (A.A.); haqqmr@gmail.com (Q.M.R.H.)

* Correspondence: atasleem@gmail.com (A.T.J.); inhochoi@ynu.ac.kr (I.C.); Tel.: +82-53-810-3589 (A.T.J.); +82-53-810-3011 (I.C.)

† These authors contributed equally to this work.

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.

5. 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 nitrate 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 nitrate 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.

<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3249911/> (Open Access Article)

<http://europepmc.org/articles/PMC3249911>

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.

Pharmacogn Rev. 2010 Jul-Dec; 4(8): 118–126.

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Free radicals, antioxidants and functional foods: Impact on human health

V. Lobo, A. Patil, A. Phatak, and N. Chandra

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Abstract

Go to: 

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.

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

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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

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CONCLUSION

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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.

<http://www.ncbi.nlm.nih.gov/pubmed/11018744>

<http://www.sciencedirect.com/science/article/pii/S1383574200000508>

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.



Mutation Research 463 (2000) 247–283



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Mammalian class theta GST and differential susceptibility to carcinogens: a review

Stefano Landi*

Environmental Carcinogenesis Division, US Environmental Protection Agency, Research Triangle Park, NC, 27711, USA

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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

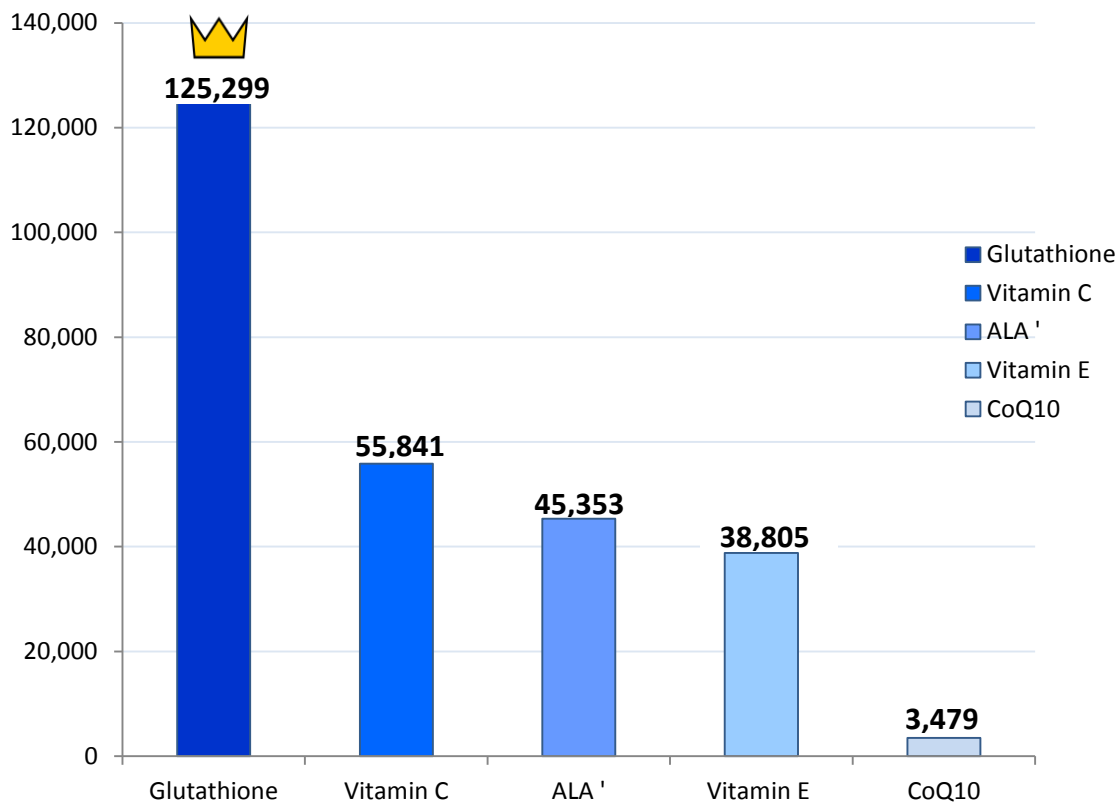
of cancer via the GSTT1-1 pathway. **Because GSTT1-1 is polymorphic in humans, with about 20% of Caucasians and 80% of Asians lacking the enzyme**, the relationship between the phenotype and the incidence of cancer has been investigated extensively in order to detect GSTT1-1-associated differential susceptibility 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.

RESEARCH STUDIES COMPARING GLUTATHIONE TO OTHER ANTIOXIDANTS



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* Pubmed.gov is the online medical science library of the U.S. Government.

' ALA (Alpha-Lipoic Acid)

In the past several months I have been working with Chinese associates in various Max markets. In many of my phone conversations I have received many questions relating to the Max products and in particular RiboCeine. To assist these associates and others I have compiled a listing and summary of studies that involved RiboCeine and have been published in scientific journals. I have provided the scientific abstract from www.Pubmed.gov as well as a summary of the conclusions. I sincerely thank Dr. Scott Nagasawa for his encouragement, his guidance and his assistance. I hope this booklet is of help to all the Max Associates. As new material becomes available I will attempt to add to meaningful summaries.

Jennifer Tsai

April 2016
