

ROS, Pathology, and the Antioxidant Minerals

Much has been reported concerning the effects of ROS (reactive oxygen species) on biological functions. Reactive oxygen species, like the superoxide anion (O_2^-), hydrogen peroxide, and peroxynitrite play major biological roles. At low concentrations, ROS act as mediators or modulators of cell signaling and contribute to other key functions, regulating the activity of transcription factors and gene expression. Transcription factors regulate the binding of RNA polymerase to DNA and as a result control the subsequent transcription of DNA to Messenger RNA and eventually the specific proteins. Transcription factors contain DNA binding domain (DBD) and often contain a second domain that senses external signaling to the rest of the transcription complex, resulting in up or down regulating of gene expression. The DBD and signal sensing domains can work together to regulate gene expression. However, it has been observed that high levels of ROS contribute to a variety of biological dysfunction, and are seen to be present in:

- Hypertension
- Atherosclerosis
- Diabetes Mellitus
- Arthritis
- Inflammatory Bowel Disease
- Alzheimers
- Infectious Disease
- Other

These pathological states and many others all have inflammation at their roots. The superoxide anion is the major culprit seen in the ROS category. The body has been seen to have three isoforms of the enzyme superoxide dismutase (SOD) that play their various roles in countering the superoxide anion and other forms of ROS. They are:

1. Cytosolic (copper-zinc SOD, SOD-1)
2. Manganese SOD (Mn SOD, SOD-2)
3. Extracellular copper-zinc SOD (EC-SOD, SOD-3)

All of the SOD forms catalyze the same reaction:



Hydrogen peroxide, H_2O_2 is also a reactive oxygen species, and it has both positive and negative impacts, which require proper control to avoid the negative ROS impact of this species. In Figure 1, a schematic illustration of the subcellular localization of the three SOD isoforms is depicted, giving a visual of where these various SOD forms exist and work in relation to one another.

Superoxide is produced by the mitochondria, a variety of oxidases (NADPH oxidase, xanthine oxidase, cyclooxygenase, lipoxygenase, etc.), as well as autooxidation of certain molecules. Superoxide anion plays into the formation of other ROS, such as peroxynitrite ($ONOO^-$) and hydrogen peroxide, which can cause further troubles and require additional enzyme handling. In Figure 2, the schematic shows the

interrelationships of the various ROS and NO (nitric oxide).

As depicted, superoxide can cause direct cytotoxic damage in itself. In addition, it can react with nitric oxide to form the potent oxidant peroxynitrite. Peroxides, if not degraded by glutathione peroxidase (GPx) or catalase also form highly reactive hydroxyl radicals.

Figure 1.

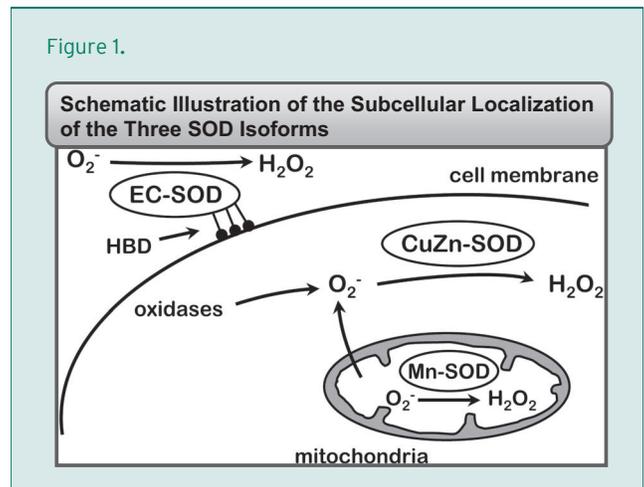
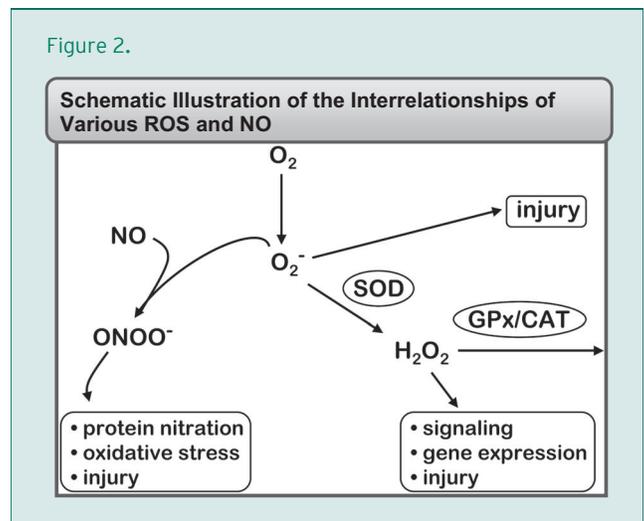


Figure 2.



Nitric Oxide (NO) is known to react with superoxide (O₂⁻) at a rate that is three times faster than the dismutation of superoxide by the SOD enzymes. Knowing this underlines the importance of maintaining a high local concentration of SOD, since SOD is the key to decreasing the biological half life of NO. The more SOD, the shorter NO half life. Decreasing the nitric oxide half life allows for less peroxynitrite formation, which leads to less cytotoxic effects due to the peroxynitrite presence.

As depicted in Figure 1, the SOD-Superoxide reaction leads to the formation of hydrogen peroxide, and as with other ROS, hydrogen peroxide is required for certain positive effects, however, too much can be toxic, and is known to cause a variety of vascular damage. More recent ROS research has now started to tell more specifically what each of the different ROS species can cause, as well as giving us more insight into the role of the individual SOD isoforms. Overall, the control of superoxide impact requires effective levels of SOD-1, SOD-2, SOD-3, as well as glutathione peroxidase and catalase. In Figure 3, the trace minerals responsible for maintaining effective levels of these five antioxidant enzymes are depicted. These trace minerals are responsible for our defense against ROS and the many pathologies associated with prolonged or elevated levels of reactive oxygen species.

supplies from 2-12% of the total SOD activity, and SOD-3 (extracellular copper-zinc SOD) provides the remainder. Recent animal studies have provided findings that give more detail on the impact of a deficiency in the various SOD isoforms. A deficiency in copper-zinc SOD (SOD-1) leads to increased levels of vascular superoxide and peroxynitrite, which has been shown to result in:

- increased myogenic tone
- increased vasoconstrictor response
- impaired relaxation of large arteries and microvessels
- increased vascular permeability
- hypertrophy of cerebral arterioles
- hypertrophy of vascular muscles

Copper-zinc SOD plays a major role in protecting against vascular disease. Manganese SOD is found in the mitochondria, and the mitochondrial electron transport chain is a major producer of superoxide. Because of the localization of Mn-SOD, this SOD isoform is considered to be the first line of defense against oxidative stress. Animal studies have shown that a complete deficiency in Mn-SOD results in death after only a few weeks with evidence of neurodegeneration, cardiac abnormalities, and extensive mitochondrial damage. Recent studies in mice indicate that Mn-SOD normally protects against vascular mitochondrial

damage and the development of atherosclerosis, as well as LDL-oxidation and caspase activation (caspases plays a major role in cell death and inflammation).

EC-SOD (SOD-3) is the only isoform of SOD that is expressed extracellularly, and it accounts for a very large

portion of total SOD activity in blood vessels. Expression of EC-SOD in vascular cells and within vessel walls can be altered in response to exercise,

growth factors, cytokines, vasoactive stimuli (like angiotensin II and NO), and homocysteine, as well as during hypertension, atherosclerosis, and diabetes. The functional importance of EC-SOD is still not clear. Studies using over expression strategies have revealed protective effects of EC-SOD on blood vessels. The effects of the three SOD isoforms are far reaching and involve themselves in the control of ROS, and thus are critical to the body's fight against a large list of ROS related pathologies. Working in conjunction with the SODs, are glutathione peroxidase (GPX) and catalase. Glutathione peroxidase upregulation maintains catalase activity at a high level in the presence of H₂O₂, and it is the GPX activity (and thus the selenium level) that appears to be the limiting factor in the peroxide detoxification system. Glutathione peroxidase prevents the auto-inactivation of catalase by hydrogen peroxide, and thus maintains catalase in its active form, allowing it to break the hydrogen peroxide down to water and oxygen. Hydrogen peroxide toxicity has been shown to cause neurological damage and resulting neurological disorders, leading to such problems as cerebral palsy, Periventricular Leukomalacia (PVL), demyelination problems and their related neurological pathologies.

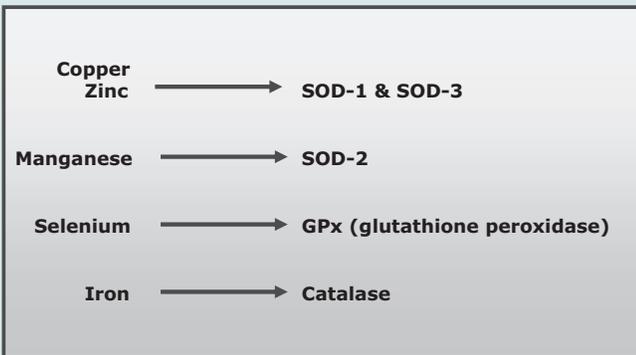
Clinical Evidence of the Pathologies Connected to SODs' Activity Level

DIFFERENTIAL MUCOSAL EXPRESSION OF THREE SUPEROXIDE DISMUTASE ISOFORMS IN INFLAMMATORY BOWEL DISEASE

L Kruidenier, et al. J Pathol 2003; 201(1):7-16.

Mucosal tissue damage and dysfunction in chronic inflammatory bowel disease (IBD) are partly caused by an enduring exposure to excessive amounts of reactive oxygen metabolites (ROMs). Although the three human isoforms of superoxide dismutase (SOD), copper/zinc (Cu/Zn)-SOD, manganese (Mn)-SOD, and extracellular (EC)-SOD, form the primary endogenous defense against ROMs, their expression levels and cellular localization

Figure 3.



Of the SOD isoforms, SOD-1 (copper-zinc SOD cytosolic) accounts for 50-80% of the body's total SOD activity, while the SOD-2 (manganese SOD mitochondrial)

in IBD mucosa are largely unknown. The present study used enzyme-linked immunosorbent assays (ELISAs), spectrophotometric activity assays, and immunohistochemistry to evaluate the protein concentration, enzymatic activity, and distribution of Cu/Zn-, Mn-, and EC-SOD in paired inflamed and non-inflamed mucosal resection specimens of patients with Crohn's disease (CD) or ulcerative colitis (UC) and compared these with the levels obtained in normal control mucosa. Gut mucosal SOD isoform expression was found to be differentially affected in IBD patients, without major differences between CD and UC. A marked step-wise increase in Mn-SOD protein levels was observed in non-inflamed and inflamed IBD mucosae, whereas the Cu/Zn-SOD content decreased with inflammation. EC-SOD was only found in low amounts, which tended to be decreased in IBD patients. Immunohistochemical evaluation confirmed these observations. Mn-SOD and Cu/Zn-SOD were both predominantly expressed in intestinal epithelial cells and the percentage of epithelial cells positive for Mn-SOD was considerably increased in IBD, whereas epithelial Cu/Zn-SOD expression was much less affected. Within the lamina propria, SOD expression was much lower. Cu/Zn-SOD and Mn-SOD were prominently present in neutrophils and macrophages, and EC-SOD was mainly localized in small vessels, stromal cells, and neutrophils. The percentage of lamina propria cells positive for Cu/Zn-, Mn-, or EC-SOD was not affected by inflammation. Enzyme activity measurements showed consistent results for Cu/Zn-SOD and EC-SOD, but the activity of Mn-SOD did not concordantly increase with the immunological assessments, which may indicate that a proportion of the Mn-SOD in IBD is present in an enzymatically inactive form. This study reveals remarkable changes in the expression levels of the three SOD isoforms in IBD, particularly in the epithelium. Disturbances in the carefully orchestrated mucosal antioxidant cascade may contribute to the induction and perpetuation of intestinal inflammation in IBD, and may have important implications for the development of antioxidant treatment of IBD patients.

SUPEROXIDE DISMUTASES IN THE

LUNG AND HUMAN LUNG DISEASES

VL Kinnula ; JD Crapo.

Am J Respir Crit Care Med, 2003 ; 167(12) :1600-19.

The lungs are directly exposed to higher oxygen concentrations than most other tissues. Increased oxidative stress is a significant part of the pathogenesis of obstructive lung diseases such as asthma and chronic obstructive pulmonary disease, parenchymal lung diseases (e.g., idiopathic pulmonary fibrosis and lung granulomatous diseases), and lung malignancies. Lung tissue is protected against these oxidants by a variety of antioxidant mechanisms among which the superoxide dismutases (SODs) are the only ones converting superoxide radicals to hydrogen peroxide. There are three SODs: cytosolic copper-zinc, mitochondrial manganese, and extracellular SODs. These enzymes have specific distributions and functions. Their importance in protecting lung tissue has been confirmed in transgenic and knockout animal studies. Relatively few studies have been conducted on these enzymes in the normal human lung or in human lung diseases. Most human studies suggest that there is induction of manganese SOD and, possibly, extracellular SOD during inflammatory, but not fibrotic, phases of parenchymal lung diseases and that both copper-zinc SOD and manganese SOD may be downregulated in asthmatic airways. Many previous antioxidant therapies have been disappointing, but newly characterized SOD mimetics are being shown to protect against oxidant-related lung disorders in animal models.

ELEVATED EXPRESSION OF CU, ZN-SOD AND MN-SOD MRNA IN INFLAMED DENTAL PULP TISSUE

C. Bödör, et al.

Int Endol J, 2007 ; 40(2) :128-32.

The aim of this study was to determine the mRNA expression levels of copper-zinc superoxide dismutase (Cu, Zn-SOD) and manganese SOD (Mn-SOD) in healthy and inflamed human dental pulp tissue. Sixteen patients with symptomatic irreversible pulpitis (eight females and eight males) were selected for study. Normal healthy pulps were removed from

extracted mandibular third molar teeth from 10 systemically healthy individuals (six females and four males). QRT-PCR analysis of Cu, Zn-SOD and Mn-SOD mRNA expression was carried out in 16 cases of irreversible pulpitis and in 10 cases of systemically healthy donors. The Shapiro-Wilk's test was used to test the normality of data, whereas the Mann-Whitney U-test was used to evaluate the significance of the differences between groups. Differences in the expression levels were considered to be statistically significant for P-values <0.05. A significant increase (P < 0.05) occurred in both Cu, Zn-SOD and Mn-SOD mRNA expression in cases of irreversible pulpitis. The increase in Mn-SOD level was significantly higher (P < 0.05) than the change observed for Cu, Zn-SOD. The development of pulpitis is associated with elevated transcription of both Cu, Zn-SOD and Mn-SOD; pulp tissue inflammation generated higher Mn-SOD transcription compared with Cu, Zn-SOD.

Recent Clinical Success with Antioxidant Trace Elements Having Positive Clinical Impact on Severe Oxidative Pathology

TRACE ELEMENT SUPPLEMENTATION AFTER MAJOR BURNS MODLATES ANTIOXIDANT STATUS AND CLINICAL COURSE BY WAY OF INCREASED TISSUE TRACE ELEMENT CONCENTRATIONS

MM Berger, et al.

Am J Clin Nutr, 2007;85:1293-300.

After major burns, patients can develop nutritional deficiencies including trace element (TE) deficiencies. Various complications, such as infections and delayed wound healing, influence the clinical course of such patients. The researchers aimed to investigate the effects of large, intravenous doses of TE supplements on circulating and cutaneous TE tissue concentrations, on antioxidant status, and on clinical outcome after major burns. This was a prospective, randomized, placebo-controlled trial in 21 patients aged 35 ± 11 y (x ± SD) with burns on 45 ± 21 % of their

body surface area. Intravenous copper, selenium, and zinc (TE group) or vehicle (V group) was given with a saline solution for 14-21 d. Blood and urine samples were collected until day 20, and skin biopsy specimens were collected on days 3, 10, and 20. The age of the patients and the severity of their burns did not differ significantly between the groups. Plasma TE concentrations were significantly higher in the TE group. In burned areas, skin contents of both selenium (P = 0.05) and zinc (P = 0.04) increased significantly by day 20. Plasma and tissue antioxidant status was improved by supplementation. The number of infections in the first 30 d was significantly lower in the TE group (P = 0.015), with a median number of 2 versus 4 infections per patient in the TE and V groups, respectively, as a result of a reduction in pulmonary infections (P = 0.03). Wound healing was improved in the TE group, with lower requirements for regrafting (P = 0.02). TE supplementation was associated with higher circulating plasma and skin tissue contents of selenium and zinc and improved antioxidant status. These changes were associated with improved clinical outcome, including fewer pulmonary infections better wound healing.

TRACE ELEMENT SUPPLEMENTATION AFTER MAJOR BURNS INCREASES BURNED SKIN TRACE ELEMENT CONCENTRATIONS AND MODULATES LOCAL PROTEIN METABOLISM BUT NOT WHOLE-BODY SUBSTRATE METABOLISM

MM Berger, et al.

Am J Clin Nutr, 2007;85:1301-6.

After major burns, patients exhibit an intense catabolism, and the wounds require surgery and grafting for closure. Complications, such as weight loss and delayed wound healing, are worsened by trace element (TE) deficiencies. The researchers aimed to assess the effects of TE supplements on systemic substrate turnover and local protein metabolism during wound healing after major burns. This was a prospective, randomized, placebo-controlled trial in 21 patients aged 35 ± 11 y with burns on 45 ± 16% of their body surface area; 12 had skin biopsies performed on days 3, 10, and 20, and 10 patients underwent a

stable-isotope investigation on day 10. Intravenous copper, selenium, and zinc (TE group) or vehicle (V group) was given with a saline solution for 14-21 d. On day 10, [¹³C]phenylalanine (600-μg/kg bolus followed by 12 μg · kg⁻¹ · min⁻¹) plus 6-[²H₂]glucose and [²H₅]glycerol were infused for 6 h to determine skin protein turnover. Biopsies were performed 1 and 6 h after the start of infusion to determine [¹³C]phe-nylalanine enrichment. The patients' mean age and burn severity did not differ significantly between the groups nor between the skin investigations subgroups. Plasma TE concentrations were significantly higher in the TE group. In the burned areas, the skin contents of selenium (P = 0.02) and zinc (P = 0.03) increased by day 20. The supernatant-to-plasma ¹³C enrichment ratio in burned skin was 0.363 ± 0.094 (TE group) and 0.286 ± 0.130 (V group) after 1 h (NS) and 0.592 ± 0.153 (TE group) and 0.262 ± 0.171 (V group) after 6 h, which reflected lower catabolism in the TE group (P = 0.03). No significant differences in whole-body substrate turnover were found between the groups. TE supplementation was associated with an increased skin tissue content of selenium and zinc and with a reduction in skin protein catabolism.

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Albion Minerals Increase Antioxidant Activity

COPPER SUPPLEMENTATION EFFECTS ON ERYTHROCYTE SUPEROXIDE DISMUTASE ACTIVITIES IN MIDDLE AGED MEN AND WOMEN

RA DiSilvestro, et al.

FASEB Exp Bio 2007, 698.8, pp. A 723.

Based on the current RDAs for copper, which fall below older recommendations, fewer people show inadequate copper intake than previously proposed. However, 8 week copper supplementation (2 mg copper as copper glycinate per day from Albion Advanced Nutrition), in middle aged adults (N=35) consistently raised values for erythrocyte activities of the copper enzyme superoxide dismutase. Placebo had no effect. The copper supplementation-induced changes in erythrocyte superoxide dismutase activities correlated with

changes in two plasma copper enzyme activities, ceruloplasmin and diamine oxidase. These results suggested that in this population, copper intake was not typically high enough to maximize copper enzyme activities.

SELENIUM GLYCINATE SUPPLEMENTATION EFFECTS ON GLUTATHIONE PEROXIDASE AND PSA IN HEALTHY MIDDLE AGED MEN

RA DiSilvestro, et al.

FASEB Exp Bio 2007, 227.2, pp. A105.

It is generally assumed that middle aged, healthy men in the USA eat adequate amounts of selenium. In contrast, 5 week supplementation with 200 ug selenium as selenium glycinate (Albion Laboratories, Inc.) increased plasma and erythrocyte glutathione peroxidase activities (p<0.05 for plasma, p<0.01 for erythrocytes). Although the increases were not big, small increases in blood glutathione peroxidase activities could be indicative of bigger increases in prostate activities of this enzyme (contention based on a rat study). Consistent with this concept, selenium glycinate supplementation reduced plasma PSA values, even though the supplementation period was not overly long for a PSA study, and starting PSA values were not abnormally high. An attempt was made to see if zinc arginate supplementation could enhance the selenium effects on glutathione peroxidase activities. Zinc supplementation produced a small increase in plasma activities, as well as in markers of zinc status, but the effects were not additive when zinc and selenium supplementation were combined. In conclusion, selenium intake may not be optimal in US middle aged men, especially in regard to prostate health; no evidence was found for improved zinc status enhancing selenium function.

LONGITUDINAL CHANGES OF MANGANESE-DEPENDENT SUPEROXIDE DISMUTASE AND OTHER INDEXES OF MANGANESE AND IRON STATUS IN WOMEN

CD Davis and JL Greger.

Am J Clin Nutr 1992; 55:747-52.

The effect of dietary factor3 on manganese- dependent superoxide

dismutase (MnSOD) activity in humans not been studied. We longitudinally evaluated changes in MnSOD activity and other indices of manganese and iron status in 47 women during a 124 d supplementation study. Subjects received one of four treatments: placebo, 60 mg iron, 15 mg manganese, or both mineral supplements daily. Manganese (from Albion Advanced Nutrition) supplementation resulted insignificant increases in lymphocyte MnSOD activity and serum manganese concentrations from baseline values but no changes in urinary manganese excretion or in any indices of iron status. Oral contraceptive use and the stage of the menstrual cycle did not confound the use of lymphocyte MnSOD activity or serum manganese to monitor manganese status, but fat intake affected both indices. This work demonstrated that lymphocyte MnSOD activity can be used with serum manganese concentrations to monitor manganese exposure in humans.

Summary and Conclusions

Superoxides and other Reactive Oxygen Species are known to cause a wide range of negative biological effects in the body. Left unchecked, these ROS will lead to a substantial number of pathologies: arthritis, atherosclerosis, hypertension, diabetes mellitus, neurological problems, cardiac problems, and more. The body has a complex series of antioxidant enzymes systems which are powered by a group of antioxidant trace minerals that are needed to help ameliorate the impact of these Reactive Oxygen Species. The major antioxidant enzymes include SOD-1, SOD-2, SOD-3, GPX, and catalase, and these require the trace minerals copper, zinc, manganese, selenium, and iron for their activation. Recent research has been able to give us a clearer picture of what the negative health effects are for a deficiency in the various antioxidant enzymes, and the negative impacts of

these deficiencies are both dramatic and severe. The positive impact that the antioxidant trace minerals can have on various pathological conditions has been clearly shown, and most recently, their benefit in a most severe inflammatory trauma has been demonstrated. The Albion Advanced Nutrition's antioxidant trace minerals have all been clinically researched, and as seen, they are known to have a positive impact on their respective antioxidant related enzyme systems' level of activity. To develop products that can have a positive impact on the body's antioxidant power, Albion's copper, zinc, manganese, and iron TRAACS™ brand of mineral chelate should be a primary component, as well as its Selenium Glycine Complex. All have proven bioavailability and enzyme level boosting power.

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