Glutathione, Orthomolecular Medicine, and Nutraceutical Therapy

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Abstract

It was more than 70 years ago that Linus Pauling identified sickle cell anemia as a molecular disease associated with alteration in oxygen metabolism in the red blood cell due to the monogenetic substitution of a single amino acid in hemoglobin. It's been 50 years since he first wrote about the concept of Orthomolecular Medicine, which focuses on adjusting the physiological levels of molecules with nutrient-derived precursors (now termed *nutraceuticals*) to promote optimal health. We now see these concepts being applied in the nutraceutical management of sickle cell anemia using glutamate as a precursor of glutathione and other conditions associated with oxidative stress.

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Nutrigenomics is a field that emerged during the first years of the 21st century. It came about when fast-paced discoveries in both genetics and nutrition intersected, and a veritable 'big bang' led to new and exciting research pursuits. The past decade has seen this field continue to evolve. The new reality is a better understanding of how to therapeutically use specific nutrients (nutraceuticals) for the management of specific metabolic conditions linked to specific genetic and epigenetic characteristics.

One very interesting example of nutraceutical therapy is the modern management of a condition that was first identified more than a century ago: monogenetic sickle cell disease. In the July 5, 2022 issue of *The Journal of the American Medical Association,* an article titled "Sickle Cell Disease: A Review" was published.[1] Since 2017, three new drugs have been approved by the Food and Drug Administration (FDA) for the treatment of sickle cell disease. One of them is the amino acid nutraceutical L-glutamine. Studies show that glutamine, when used therapeutically, can decrease reactive oxygen species in red blood cells, thereby reducing pathology associated with sickling of the red cells. In a randomized clinical trial involving 230 patients with sickle cell trait genetics aged 5 years and older, it was found that glutamine supplementation, compared to placebo, reduced pain associated with sickle cell crisis by 25%, hospitalization by 33%, mean length of hospital stay from 11 to 7 days, and acute chest syndrome by 23%.[2]

To best understand the mechanism of action of glutamine in sickle cell disease, a brief review of the history and pathophysiology of this condition is necessary. In 1949, Dr. Linus Pauling, who would go on to earn the distinct honor of being a two-time Nobel Laureate, published a seminal paper in *Science* with several of his research colleagues: "Sickle Cell Anemia: A Molecular Disease."[3] What was so remarkable about this paper was that it was the first to use the term *molecular disease* to describe the complex symptom profile associated with sickle cell disease. This work also linked these symptoms to a genetic single amino acid substitution in the hemoglobin molecule in red blood cells and the way that oxygen is managed in the cell, suggesting that sickle cell disease is a result of some type of oxygen-related pathology.

Over the 20 years that followed the publication of that article, Linus Pauling and his research team were on a quest to understand how specific alterations in the genetic code of an individual could influence metabolism, and also how levels of metabolites found within cells could alter the cellular environment to improve physiological function. He concentrated much of his focus on disorders associated with neurological function, and this culminated in his development of the concept of Orthomolecular Medicine and another landmark paper published in *Science:* "Orthomolecular Psychiatry" (1968). Pauling defined *orthomolecular psychiatry* as the treatment of mental disease through the provision of the optimal molecular environment for the nervous system, especially the optimum concentrations of substances normally present in the body, many of which are dependent upon the intake of specific nutrients.[4] Now, 50 years later, the original concepts of Orthomolecular Medicine serve as a framework for understanding the therapeutic use of nutraceuticals, and we have seen hundreds of both basic

science and clinical intervention studies that validate this foundational way of thinking.[5-7]

Sickle Cell Disease and Oxidative Stress-Induced Pathology

If it was suggested in 1949 that the pathology of sickle cell disease was associated with some defect in the way that oxygen is metabolized, what has been learned about this in the intervening 70 years? The mechanism of this pathology has become much better elucidated, and we now know it is associated with oxidative stress that originates in the red blood cell. Oxidative stress is defined as the imbalance between the levels of reactive oxygen species such as peroxide, hydroxyl radical, superoxide and singlet oxygen, and intercellular antioxidant activity. Reactive oxygen species have been identified to be produced in the red blood cell in those who carry the sickle cell genetic trait. It is known that the lifestyle and dietary factors of these individuals can both activate and enhance the oxidative stress-induced pathology of sickle cell disease.[8]

To counteract the adverse effects of reactive oxygen and nitrogen species, both non-enzymatic and enzymatic antioxidant defense mechanisms have evolved. Non-enzymatic, nutrient-derived antioxidants include ascorbic acid, glutathione, tocopherols, flavonoids, carotenoids, riboflavin, niacin, selenium, and zinc. In sickle cell disease, it has been found that high levels of reactive oxygen species deplete cellular antioxidants, which in turn results in oxidant damage to adjacent tissues. Nutraceutical approaches designed to increase intracellular antioxidant levels are now being explored for the management of sickle cell disease, with particular focus on glutathione and its potential to protect against the vasculopathy, inflammation, and coagulopathy associated with this condition. L-glutamine, glycine, *N*-acetylcysteine, zinc, vitamin E tocotrienols, alpha-lipoic acid, L-carnitine, quercetin, and omega-3 fatty acids have all been evaluated as potential nutraceutical agents to improve intracellular antioxidant defenses in red blood cells.

Nutraceutical Impact on Glutathione

A 2018 randomized clinical trial published in *The New England of Medicine* indicated that in patients with sickle cell disease, dietary supplementation with doses of the amino acid L-glutamine at 0.3 grams/kilogram of body weight per day resulted in a statistically significant reduction of pain crises, as well as clinical improvement in quality of life after 48 weeks.[9]

Glutamine is a cellular substrate for the synthesis of glutathione, the master intercellular antioxidant. The successful therapeutic application of L-glutamine to drive the cellular synthesis of glutathione is consistent with the concept of Orthomolecular Medicine proposed by Linus Pauling in the 1960s. Glutathione is composed of three amino acids: glutamate, glycine, and cysteine. The therapeutic administration of these three nutraceutical precursors has been found to increase the cellular synthesis of glutathione in conditions where it is depleted as a consequence of oxidative stress or detoxification burden.[10] This molecular mode of action of L-glutamine and its potential to reduce the severity of sickle cell disease was a major factor in this molecule receiving FDA approval as a drug for the treatment of this condition.

Another important nutraceutical precursor for reducing oxidative stress and increasing cellular synthesis of glutathione is *N*-acetylcysteine (NAC). A 2012 clinical trial found that 1200 mg/day of supplemental NAC, as a bioavailable source of L-cysteine necessary for the synthesis of glutathione, resulted in increased whole blood glutathione levels and a significant reduction in pain syndrome in patients with sickle cell disease.[11] This study nicely complements the Orthomolecular Medicine concept of improving metabolic function by manipulating the internal cellular environment of specific biomolecules with substances that are native to human physiology.

The third amino acid that is a component of glutathione is glycine. There is clinical evidence that supplementation with glycine, in combination with NAC in older adults, increases whole blood glutathione levels, reduces both oxidative stress and inflammation, and improves endothelial function. A 24-week open-label clinical trial at Baylor University Medical Center with older age individuals who supplemented daily with 100 mg/kg of glycine and 130mg/kg *N*-acetylcysteine found that the nutraceutical regimen was well tolerated and resulted in increased intracellular glutathione, decreased inflammation markers, improved insulin sensitivity and endothelial function, and improved strength, gait speed, cognition, and body composition.[12]

Nutraceutical Therapy, Glutathione, and Functional Health Outcomes

The demonstrated clinical value of L-glutamine nutraceutical therapy for the management of oxidative stress-related symptoms in patients with sickle cell disease raises the question as to whether other chronic conditions associated with oxidative stress would benefit from an Orthomolecular Medicine approach to increasing intracellular glutathione.

It is well known that patients infected with HIV suffer from increased oxidative stress, which results in altered metabolic function. It has recently been shown, in an open-label trial with HIV infected males, that supplementation with the glutathione precursors *N*-acetylcysteine (130 mg/kg/day) and glycine (100 mg/kg/day) led to significant improvement in insulin sensitivity, body composition and muscle strength, and increased whole blood glutathione levels.[13]

In a placebo-controlled study of 83 older-age individuals (average age 72), it was found that L-glutamine supplementation (0.3 gm/kg/day) along with daily

programmed exercise resulted in significant improvements in age-related physiological parameters after 30 days.[14] These benefits included a reduction in inflammatory markers and increased intracellular glutathione levels.

In a double-blind study with 44 older-age women (age 60-80), it was found that 10 g/day of supplemental L-glutamine resulted in significant clinical improvement in strength and power of knee muscles, glycemic control, and increased plasma antioxidant defense levels.[15] The combination of a programmed exercise regimen coupled with the L-glutamine supplementation resulted in the greatest improvements in whole blood glutathione levels and improved clinical function. Markers for oxidative stress were significantly reduced in this group, a fact that once again connects L-glutamine supplementation with improved whole blood glutathione levels and reduced indices of biological aging.

The results from these clinical trials are encouraging. More recently, the Translational Metabolism Unit at the Baylor College of Medicine initiated and completed a pilot trial evaluating the impact of glycine and *N*-acetylcysteine supplementation in patients with type 2 diabetes. In this study, 10 patients with poorly controlled type 2 diabetes and hemoglobin A_{1c} levels from 8-10% and 10 control patients without type 2 diabetes were administered 100 mg/kg/day of glycine and 100 mg/kg/day of *N*-acetylcysteine for two weeks. Mitochondrial function was evaluated in the participants before and after the intervention period. It was found that supplementation with the glutathione precursors significantly improved fasted mitochondrial function by 30%, decreased insulin resistance by 22%, and lowered plasma free fatty acids by 25%. The investigators are clear in summarizing the results of this study: "These results provide proof-of-concept that GlyNAC [glycine and *N*-acetylcysteine] supplementation could improve mitochondrial dysfunction and IR [insulin resistance] in patients with T2D [type 2 diabetes] and warrant additional research."[16]

Concluding Thoughts

It was more than 70 years ago that Linus Pauling identified sickle cell anemia as a molecular disease associated with alteration in oxygen metabolism in the red blood cell due to the monogenetic substitution of a single amino acid in hemoglobin. It's been 50 years since he first wrote about the concept of Orthomolecular Medicine, which focuses on adjusting the physiological levels of molecules with nutrient-derived precursors (now termed *nutraceuticals*) to promote optimal health. Today—all these years and decades later—we are witnessing the advance of this concept across a spectrum of health-related problems, especially those that have their roots in genetic individuality. The use of tetrahydrobiopterin to improve the function of those who are born with the monogenetic disorder phenylketonuria (PKU) is just one example.[17] Tetrahydrobiopterin is the cofactor necessary for the function of phenylalanine hydroxylase, and its supplementation as nutraceutical has been found to help lower blood phenylalanine levels in individuals with PKU.

The successful application of the Orthomolecular Medicine concept links to many variables beyond that of the specific nutraceutical itself. It includes the full spectrum of lifestyle and environmental factors that influence genetic and epigenetic expression, including activity level, stress, toxic exposures, dietary composition, and chronobiology. We can see this in a study of healthy men and women who participated in a diet and lifestyle program focused on improving neurological function and reducing oxidative stress. In this double-blind, placebo-controlled study, 63 healthy females and males aged 40-60 participated in a 21-day trial. The participants were on a calorie-restricted diet that was supplemented with a complex mixture of nutrients that had been shown in various studies to favorably impact oxidative stress and stimulate the cellular synthesis of glutathione. All participants in both the treatment and placebo groups had initial and final brain NMR scans performed to evaluate brain metabolic function. The results of the study after six weeks were very encouraging, in that the findings indicated that the complex nutritional supplement, when delivered in a calorie-controlled program, resulted in significant improvement in neurochemistry, which was indicative of enhanced brain glutathione concentrations and a reduction in oxidative stress. [18]

This study reminds us that while it is important to recognize that certain nutrients may have valuable nutraceutical applications for specific health conditions, the impact of the composition of the whole diet and lifestyle play very important roles in determining the successful outcomes of Orthomolecular Medicine interventions.

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