Spirulina Rising: Microalgae, Phyconutrients, and Oxidative Stress

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Abstract

Oxidative stress provokes the development of many common diseases and contributes to the aging process. Also, oxidative stress is a critical factor in common vascular disorders and type 2 diabetes. It plays a role in neurodegenerative disorders, such as Alzheimer's disease, Parkinson's disease, amytrophic lateral sclerosis, multiple sclerosis, and cancer. Oxidative stress contributes to the healthy regulation of cell function. However, excessive oxidative stress results in pathological processes. Antioxidant vitamins have a limited influence on oxidative stress as most oxidative stress results from the cellular production of superoxide. Bilirubin protects cells from oxidative stress, and inhibits NOX. PhyCB, a component of the microalgae Spirulina, shares a similar structure with biliverdin, a biosynthetic precursor of bilirubin. Thus, the oral administration of PhyCB, phycocyanin, or whole Spirulina shows promise for preventing and or treating human disorders that have resulted from excessive oxidative stress.

Keywords: Microalgae; Oxidative Stress; Phycocyanobilin; Phyconutrients; Singlet Oxygen; Spirulina; Superoxide

Abbreviations

DHA: Docosahexaenoic Acid; HO-1: Heme Oxygenase-1; O_2: Oxygen; PhyCB: Phycocyanobilin

Introduction

To understand how phyconutrients, found abundantly in microalgae, can provide preventive and therapeutic effects, it is fundamental to understand how oxidative stress triggers or contributes to the development of many common diseases—and how microalgae, such as Spirulina can help inhibit oxidative stress in the human body.

Oxidative stress is the core driver in a range of health disorders and contributes to the physiological decrements of aging. Oxidative stress plays a key role in the induction of all types of vascular disorders, including atherosclerosis, high blood pressure, ventricular hypertrophy, congestive heart failure, atrial fibrillation, vascular erectile dysfunction, and the small vessel complications of diabetes (leading to blindness, kidney failure, and peripheral nerve damage). In atherosclerotic individuals, oxidative stress contributes to heart attacks, strokes, and the heart or brain damage induced by these events. Oxidative stress not only exacerbates the complications of diabetes but also induces and sustains the failure of blood sugar control that is characteristic in type 2 diabetes. Common neurodegenerative disorders, such as Alzheimer's disease, Parkinson's disease, amytrophic lateral sclerosis, and multiple sclerosis, are driven by self-sustaining inflammation in the brain or peripheral nerves, in which oxidative stress plays a chief role.

Oxidative stress is a mediator of mutagenic DNA damage of stem cells, which is a primary cause of cancer. It also promotes the conversion of precancerous tissues into overt cancer and the aggressive growth of many cancers by boosting cancer-stimulating growth factors. Oxidative stress contributes to the cartilage degeneration characteristic of osteoarthritis and participates in the tissue damage associated with many autoimmune conditions, such as rheumatoid arthritis. Much evidence points to oxidative stress as a driver of age-related macular degeneration, the most common cause of partial blindness in the elderly. Oxidative stress participates in chronic liver damage associated with hepatitis, alcohol abuse, and obesity. Oxidative stress in immune cells plays a role in the destruction of bacteria. Other disorders linked to oxidative stress include asthma, preeclampsia, and pulmonary fibrosis.

However, not all oxidative stress is harmful or damaging to the human body; in modest amounts, it contributes to the appropriate regulation of cell function. Nonetheless, excessive oxidative stress contributes to the lethality of influenza and septic shock. The most common types of heart disease, hypertension, heart attack, stroke, diabetes, cancer, neurodegenerative conditions (including dementia, osteoarthritis, macular degeneration, complications of autoimmunity, and chronic liver damage) have their basis in excessive oxidative stress. Oxidative stress is not the only metabolic factor provoking these health disasters, but it plays a significant role in the prevention or amelioration of specific disorders and diseases.

In healthy people, oxidative stress can have a limiting impact on physical performance. For example, it can limit endurance during prolonged moderate-intensity exercise. Also, cerebral oxidative stress likely plays a role in the gradual decline in mental acuity that typically accompanies aging.

Discussion

Limitations of antioxidant vitamins

Vitamin C, vitamin E, and beta-carotene are commonly known antioxidants and are considered complementing factors to good health. Except for macular degeneration, clinical trials evaluating these nutrients have had disappointing outcomes, failing to demonstrate heart attack or cancer prevention [1–3]. These research findings seem discordant with the evidence that oxidative stress is a principal mediator of numerous disorders; thus questioning whether antioxidant approaches have any potential for health protection.

Antioxidant vitamins have a limited impact on the downstream metabolic consequences of oxidative stress. Most oxidative stress results from the cellular production of superoxide. Superoxide is converted, either spontaneously or enzymatically, to the unstable compounds hydrogen peroxide and peroxynitrite, which can break down to yield "free radicals" that attack any molecule in their vicinity. The antioxidant vitamins do not inhibit this downstream process. They do, however, donate electrons; and in doing so, they can initiate repair of molecular damage by free radicals. Nevertheless, their efficacy in this regard is limited.

Vitamin E can only repair damage to fat-soluble compounds residing in cellular membranes; it cannot counteract the negative impact of free radicals on water-soluble compounds in the cell's interior (cytoplasm). Moreover, vitamin E is inefficacious as a membrane antioxidant. Most people receive adequate amounts of vitamin E from their standard diet, but supplementing with this nutrient provides only modest further protection to cell membranes [4].

Vitamin C provides essential antioxidant protection to the interior (cytoplasm) of human cells. In people consuming a diet containing some fruits and vegetables, the vitamin C content of cells is nearly saturated. Thus, the ingestion of megadoses of this vitamin will not achieve significant elevation of cellular vitamin C levels [5,6]. Beta-carotene, as a source of vitamin A, can protect cell membranes from oxidative stress induced by UV light exposure (singlet oxygen), but it fails to protect the cell cytoplasm. In most respects, it is less effective than vitamin E as a membrane antioxidant.

The adverse consequences of oxidative stress result from the impact of hydrogen peroxide on cellular signaling pathways, which the vitamin antioxidants cannot prevent; the hydrogen peroxide effects do not entail the production of free radicals.

Limiting the downstream flow of oxidative stress

One strategy to overcome or limit oxidative stress is to suppress the production of superoxide. If superoxide production is controlled, all of the downstream consequences of oxidative stress will be attenuated.

Statin drugs and drugs that antagonize the bioactivity of the hormone angiotensin II (ACE inhibitors and angiotensin receptor blockers) demonstrate the ability to inhibit superoxide production, to a degree; contrary to antioxidant vitamins that consequently are not as protective as these specific drugs [1].

How is superoxide formed?

The two primary cellular loci of superoxide production are mitochondria and a group of membrane-bound enzyme complexes, known as NADPH oxidase (NOX). When these primary sources react to high oxidative stress, damage to the enzymes nitric oxide synthase and xanthine oxidase results in secondary sources of oxidative stress: a feed-forward mechanism in which oxidative stress gives rise to more oxidative stress. Moreover, oxidative damage to the mitochondrial membrane can stimulate the production of superoxide. Oxidative stress can induce the synthesis of specific proteins that constitute NOX. In counteracting these adverse outcomes, oxidative stress enhances the production of antioxidant enzymes and the cellular antioxidant glutathione. These two compounds work to eliminate superoxide and hydrogen peroxide and repair the damage that the latter causes.

Thus, NOX and mitochondria need to be regulated if superoxide production is to be maintained at a safe level. Superoxide production cannot be eliminated entirely as mitochondria continuously generate small amounts of superoxide as a by-product of their crucial role as an ATP generator. However, damage to the mitochondrial membranes—most commonly caused by oxidative stress or specific pro-inflammatory hormones [7]—can amplify mitochondrial superoxide production to dangerous levels. The beneficial role of NOX in the human body is to generate superoxide in immune cells (phagocytes) which aids in the destruction of ingested microorganisms. Also, in a variety of cell types, NOX stimulates small amounts of superoxide in a controlled manner so that the resulting production of hydrogen peroxide can modulate cellular signaling mechanisms. Unfortunately, in a great many health disorders, the level and activation state of NOX become highly elevated in affected tissues. The resulting oxidative stress makes the disorder worse or causes it to occur [8].

Since it is implausible to prevent superoxide production entirely, and given the beneficial physiological role of NOX in infection control and cellular signaling mechanisms, strategies are needed that can suppress excess superoxide production and overactive NOX. Microalgal phytonutrients appear to have noteworthy potential in these regards.

Bilirubin and Phycocyanobilin versus NOX

The microalga Spirulina (Arthrospira platensis) is rich in phycocyanobilin (PhyCB). In microalgae, PhyCB is attached to the protein phycocyanin and functions in harvesting light energy (similar to chlorophyll). Light-harvesting is crucial to the growth and survival of microalga; thus, the PhyCB content of Spirulina is exceptionally high, averaging about 0.6 % of Spirulilna's dry weight. For micronutrients, this percentage is a comparatively high concentration.

PhyCB is a close structural analog of bilirubin, a compound produced in the human body that performs vital antioxidant functions. This similarity supports PhyCB as a potential and powerful antioxidant. Spirulina synthesizes PhyCB from biliverdin (a compound found in humans as the biosynthetic precursor of bilirubin). Bilirubin can function as a proficient scavenger of free radicals (like antioxidant

vitamins). Bilirubin, bound to the protein albumin in the bloodstream, protects the plasma from oxidative stress [9,10]. Bilirubin functions as a highly-potent inhibitor of certain forms of NOX [8,11,12]. There are several different forms of NOX [13]. It is not yet clear whether bilirubin can inhibit all NOX forms, but it inhibits some of the most common forms of NOX involved in disease induction.

Bilirubin participates in a feedback mechanism that protects cells from oxidative stress. When cells are producing excessive amounts of superoxide and become oxidatively-stressed, there is a corresponding increase in the production of the enzyme, heme oxygenase-1 (HO-1). HO-1, in turn, breaks down heme (the organic iron complex found in many proteins including hemoglobin). The products of this degradation of heme are an iron atom, carbon monoxide, and biliverdin. It is paradoxical that, in high concentrations, carbon monoxide can cause asphyxiation; in low concentrations (as produced by HO-1) carbon monoxide demonstrates anti-inflammatory and cell-protective properties that are often beneficial, particularly in oxidatively stressed tissues.

Biliverdin is converted, enzymatically, to bilirubin within the cell. This bilirubin can then inhibit NOX, which is a key source of the oxidative stress that triggers HO-1 synthesis. Cellular oxidative stress prompts increased production of HO-1. This increased production of HO-1 leads to the synthesis of bilirubin. The resultant bilirubin inhibits NOX, resulting in the amelioration of oxidative stress [14].

Bilirubin in its free form is highly insoluble and the intracellular concentrations generated by HO-1 are extremely low—about 50,000 times lower than that of other key intracellular oxidant scavengers, such as vitamin C and glutathione [15]. Thus, bilirubin's role as an intracellular scavenger of free radicals is limited; its importance stems from its ability to inhibit NOX. In the bloodstream, free bilirubin binds with high affinity to albumin, which effectively renders it soluble. Albumin-bound bilirubin is found in high concentrations in the blood; hence, in the bloodstream, it performs essential oxidant scavenging activities.

Bilirubin can inhibit NOX. Epidemiological studies have found that people with relatively high blood levels of bilirubin are less likely to develop cardiovascular disorders, cancers, diabetes, or diabetic complications [16–19] than people with relatively low blood bilirubin levels. Free bilirubin in the blood is in equilibrium with bilirubin in the cells. It can be transported from the cells into the bloodstream, and vice versa. Thus, a high level of bilirubin in the blood should translate into a higher capacity to inhibit NOX in the cells. Elevated NOX levels have been linked to cardiovascular disorders, cancer, diabetes, and diabetic complications, which are rarer in people with high bilirubin levels.

Specific individuals express elevated free bilirubin blood levels due to the inheritance of a variant form of a gene coding for a protein in the liver that links bilirubin to a sugar molecule, expediting its excretion in the bile. People with this syndrome (known as Gilbert syndrome) produce lesser amounts of this protein, resulting in higher amounts of free bilirubin accumulating in the blood and tissues. Those with Gilbert syndrome are known to be at low risk for heart disease. Dr. Toyoshi Inoguchi, a Japanese diabetologist, conducted an epidemiological study in which he reviewed the records of nearly one hundred diabetics who also had Gilbert syndrome and compared the findings to the records of several thousand diabetics not known to have this syndrome. After making statistical adjustments regarding the duration and severity of diabetes in the two groups, he remarkably found that the diabetics with Gilbert syndrome were two-thirds less likely to experience heart disease, develop kidney damage, or lose their eyesight [17].

The prospect of inhibiting diabetic complications by the administration of bilirubin is promising. However, bilirubin is highly-insoluble; thus, oral administration would be ineffective. However, its precursor, biliverdin, is soluble and can be absorbed from the gastrointestinal tract to some degree. In another study, Dr. Inoguchi demonstrated that, in diabetic mice, oral administration of biliverdin could completely inhibit glomerulosclerosis that leads to kidney failure in diabetic mice [20]. The kidneys of diabetic mice typically display oxidative stress and an increase in NOX levels. It is likely that NOX-induced oxidative stress was responsible for the increase in NOX levels seen in the kidneys of these diabetic mice. Apparently, from Dr. Inoguchi's study, bilirubin prevented that increase.

These findings suggest that biliverdin might have the potential for not only controlling the complications of diabetes but also the vast range of disorders linked to excessive NOX activity. However, there are no rich, natural sources of biliverdin, and this complex compound is costly to synthesize.

The structural similarity between PhyCB and biliverdin

PhyCB and biliverdin are structurally similar (Figure 1). There are only small differences at the ends of the molecules, which have little impact on their overall configuration. This resemblance is no coincidence as biliverdin is the precursor for PhyCB synthesis in Spirulina and other microalgae that contain it.
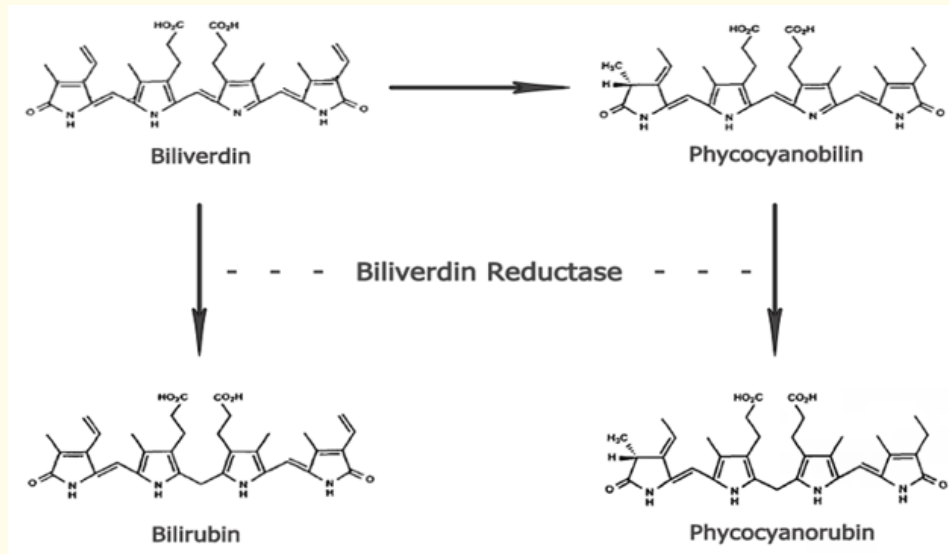

Figure 1: Comparative structural configurations of biliverdin to phycocyanobilin and bilirubin to phycocyanorubin.

Biliverdin does not inhibit NOX; bilirubin does. The enzyme that converts biliverdin to bilirubin, known as biliverdin reductase, causes a significant change in the three-dimensional structure of the molecule, accounting for bilirubin's poor solubility. Thus, although PhyCB looks similar to bilirubin "on paper" in two dimensions, its three-dimensional structure is quite different. However, the enzyme biliverdin reductase—found in virtually all human cells—efficiently converts PhyCB to phycocyanorubin, having an almost identical structure to bilirubin [21]. Thus, it is posited that phycocyanorubin could mimic the NOX-inhibitory activity of bilirubin.

In a series of studies conducted by Cuban scientists, in which oral administration to rodents of phycocyanin (the Spirulina protein that carries attached PhyCB) showed potent anti-inflammatory and cell-protective properties in a broad range of models of inflammation [22]. These models included induced paw or ear inflammation, arthritis, colitis (colon inflammation), liver damage, brain injury, and septic shock. Researchers in Mexico City and India have demonstrated that orally administered whole Spirulina showed anti-inflammatory activity in rodents [23–26]. While these models of inflammation and tissue damage are disparate, they are united by the over activation of NOX, contributing to the concomitant oxidative stress.

Dr. Inoguchi carried out futher studies comparing the impact of biliverdin and PhyCB on the NOX activity of a range of cultured human cells. Biliverdin and PhyCB were comparatively helpful for inhibiting NOX activity in these cells (Figure 2). Presumably, the activity of PhyCB, in this regard, stemmed from its intracellular conversion to phycocyanorubin, as the activity of biliverdin stems from its conversion to bilirubin. The intensity of inhibition increased as the concentration of biliverdin or PhyCB increased until a plateau was reached, at which point NOX inhibition was nearly complete.
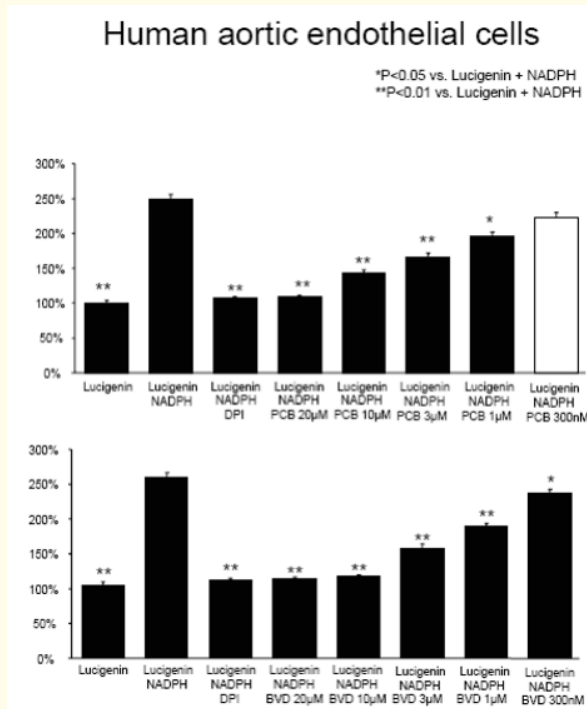
Figure 2: *Results of Dr. Inoguchi's study on the impact of biliverdin and PhyCB on the NOX activity of a range of cultured human cells. Note. Biliverdin and PhyCB were almost identically effective for inhibiting NOX activity in these cells.*

More recently, Dr. Inoguchi demonstrated that orally-administered phycocyanin (free PhyCB) had precisely the same protective impact on the kidneys of diabetic mice that oral biliverdin showed [27]. One study seemed to confirm phycocyanin's beneficial effects. In hamsters fed a high-fat and high-cholesterol diet, the oral administration of phycocyanin or whole Spirulina was shown to markedly inhibit early atherosclerosis (by more than 80%), while decreasing NOX expression and oxidative stress in the heart. The researchers noted that phycocyanin "powerfully prevents the development of atherosclerosis" in this model [28]. (In contrast, vitamin E—which has been researched extensively—had no impact in this model.) A recent Cuban study showed that oral phycocyanin was highly protective in the mouse model for multiple sclerosis (known as experimental autoimmune encephalomyelitis) [29]. Moreover, oral phycocyanin or Spirulina proved protective in two rodents studies involving inflammatory damage to the central nervous system, suggesting that orally-administered PhyCB has access to the brain (whereas, many drugs do not). Thus, PhyCB may have utility for the control of neurodegenerative disorders and stroke damage [23,30].

Conclusion

The implications of this research for human health are significant, with the assumption that humans metabolize oral PhyCB similar to the metabolic pathways of rodents. The oral administration of PhyCB, phycocyanin, or whole Spirulina shows the potential to prevent or treat a range of human disorders—in which overactivation of NOX (or at least those forms of NOX production inhibitable by bilirubin) plays a key pathogenic role. This pathological overactivation of NOX may contribute to a high proportion of the degenerative disorders that afflict humankind today, and to which PhyCB, phycocyanin, or whole Spirulina could be beneficially applied.

Conflict of Interest Statement

The authors declare that this paper was written in the absence of any commercial or financial relationship that could be construed as a potential conflict of interest, at the time of its research and writing. Subsequently, Dr. Mark F. McCarty has become co-inventor and co-owner of U.S. and E.U. patents on the use of PhyCB oligopeptides as nutraceuticals, and holds an E.U. patent on the use of PhyCB for the prevention and control of diabetic glomerulosclerosis.

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Supplementary Note

Parts of this paper were previously made available in a booklet entitled, *A Guide to Health-Protective Microalgal Phyconutrients,* posted on the Capitalife, Inc. (USA) website, and used with permission.

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