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Perspective

Clinical Potential of *Spirulina* as a Source of Phycocyanobilin

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ABSTRACT Recent research reveals that free bilirubin functions physiologically as a potent inhibitor of NADPH oxidase activity. The chromophore phycocyanobilin (PCB), found in blue-green algae and cyanobacteria such as *Spirulina*, also has been found to be a potent inhibitor of this enzyme complex, likely because in mammalian cells it is rapidly reduced to phycocyanorubin, a close homolog of bilirubin. In light of the protean roles of NADPH oxidase activation in pathology, it thus appears likely that PCB supplementation may have versatile potential in prevention and therapy—particularly in light of rodent studies demonstrating that orally administered *Spirulina* or phycocyanin (the *Spirulina* holoprotein that contains PCB) can exert a wide range of anti-inflammatory effects. Until PCB-enriched *Spirulina* extracts or synthetically produced PCB are commercially available, the most feasible and least expensive way to administer PCB is by ingestion of whole *Spirulina*. A heaping tablespoon (about 15 g) of *Spirulina* can be expected to provide about 100 mg of PCB. By extrapolating from rodent studies, it can be concluded that an intake of 2 heaping tablespoons daily would be likely to have important antioxidant activity in humans—assuming that humans and rodents digest and absorb *Spirulina*-bound PCB in a comparable manner. An intake of this magnitude can be clinically feasible if *Spirulina* is incorporated into "smoothies" featuring such ingredients as soy milk, fruit juices, and whole fruits. Such a regimen should be evaluated in clinical syndromes characterized and in part mediated by NADPH oxidase overactivity in affected tissues.

KEY WORDS: • ***antioxidant*** • ***bilirubin*** • ***biliverdin*** • ***heme oxygenase*** • ***inflammation*** • ***NADPH oxidase*** • ***phycocyanin*** • ***phycocyanobilin*** • **Spirulina** • ***zeaxanthin***

NADPH OXIDASE AS A MEDIATOR OF PATHOLOGY

IN A HIGH PROPORTION of non-infectious pathologies, NADPH oxidase becomes activated in the affected tissues, and the resulting generation of oxidants either mediates or exacerbates the pathology. Thus, as cited earlier,[1] overactivity of this enzyme complex appears to play a role in a host of vascular disorders, including atherogenesis, hypertension, left ventricular hypertrophy, aneurysms, and ischemia-reperfusion injury; the insulin resistance associated with obesity; the major complications of diabetes; neurodegenerative disorders such as Alzheimer's and Parkinson's diseases; various autoimmune conditions, including rheumatoid arthritis and scleroderma; allergy and asthma; hepatic fibrosis; ultraviolet-mediated skin damage; and the cartilage loss associated with osteoarthritis. Oxidant stress generated by NADPH oxidase also sometimes contributes to cancer initiation, boosts growth factor activity in some cancers, and is a mediator of the angiogenic process. Thus, it is reasonable to suspect that tolerable clinical strategies for down-regulating NADPH oxidase activity may have a remarkably versatile potential in both preventive and therapeutic medicine. Indeed, the surprising range of benefits associated with statin and angiotensin converting enzyme inhibitor therapies likely reflects, in part, their ability to suppress NADPH oxidase activation in certain tissues.[2,3]

Fortunately, Nature has equipped us with feedback mechanisms that help to moderate the generation of oxidant stress. In particular, recent research has established that free bilirubin, in the low nanomolar concentrations that prevail intracellularly, functions physiologically as a potent and highly specific inhibitor of NADPH oxidase.[4–6] Intracellular oxidant stress induces expression of heme oxygenase-1, which in turn generates bilirubin from heme via biliverdin[7,8]; this mechanism provides feedback control of the oxidant stress mediated by NADPH oxidase. Bilirubin's suppressive impact on NADPH oxidase activity likely explains the growing epidemiological literature that associates increased serum bilirubin, or high-expression polymorphisms of heme

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oxygenase-1, with diminished risk for vascular disorders, certain cancers, and various other diseases.[1,9–12]

PHYCOCYANOBILIN (PCB)—A PHYTONUTRIENT INHIBITOR OF NADPH OXIDASE

Potentially, bilirubin, or preferably its more soluble precursor biliverdin, could be used as orally administered antioxidants for prevention and control of a wide range of disorders. However, there are no rich natural sources of these compounds, which moreover are difficult and expensive to synthesize. It is therefore quite fortunate that many algae and cyanobacteria are rich in the compound PCB, a chromophore that, as a component of the holoprotein phycocyanin, aids the harvesting of light energy.[13] PCB is a biliverdin derivative that, in mammalian cells, is converted by the ubiquitously expressed enzyme biliverdin reductase to phycocyanorubin, a compound nearly identical in structure to bilirubin.[14] Recent studies by T. Inoguchi (personal communication) show that addition of either PCB or its homolog biliverdin to human cell cultures leads to potent inhibition of NADPH oxidase; this effect is dose-dependent in the low micromolar range, and near maximal at 20 μM. The fact that orally administered phycocyanin or *Spirulina* exerts potent and versatile anti-inflammatory effects in rodents[15–24] strongly suggests that ingested PCB can be sufficiently well absorbed to provide important systemic antioxidant activity. PCB's homolog biliverdin is likewise effective when administered orally.[25–27] Thus, PCB supplements, once available, may have considerable potential for health protection.

However, until PCB-enriched *Spirulina* extracts or PCB derived from bioengineered organisms[28] or chemical synthesis are commercially available, the most practical source of PCB for supplemental use is whole *Spirulina*. Phycocyanin constitutes about 14% of the total dry weight of *Spirulina*; PCB represents about 4.7% of the mass of phycocyanin.[29] It follows that about 0.66% of the dry mass of *Spirulina* is PCB. In other words, 15 g of *Spirulina*—approximately a heaping tablespoon—contains about 100 mg of PCB. The fraction of this that is absorbed in a form capable of inhibiting NADPH oxidase is unknown.

SPIRULINA AS PRACTICAL CLINICAL SOURCE OF PCB

If we make the not unreasonable assumption that absorption and metabolism of *Spirulina*-bound PCB are similar in rodents and humans, then clinically useful dose regimens of *Spirulina* can be estimated by extrapolating from regimens that demonstrate antioxidant efficacy in rodents. Such dose extrapolation can be done straightforwardly on a mg/kg basis. However, in clinical practice, dose is often adjusted by relative body surface area, which corresponds to the 2/3 power of the ratio of body weights. This latter standard evidently yields a much lower correction factor. A commonly employed compromise between these two standards is to adjust dose by the 3/4 power of the ratio of body weights; this has been found to offer a "best fit" when extrapolating various quantifiable metabolic parameters between mammalian species.[30–32] The 3/4 power standard yields a correction factor of about 80 if comparing a 200 g rat with a 70 kg human, or a factor of 450 if comparing a 20 g mouse with a 70 kg human. (In other words, if a rat receives x mg of an agent, the corresponding human dose would be $80x$ mg.)

In an extensive series of investigations, Romay and coworkers have reported that oral phycocyanin administered orally to mice and rats exerts a number of dose-dependent anti-inflammatory effects in a dose range of 50–300 mg/kg/day.[15–18] This amounts to a PCB intake of 2.35–14.1 mg/kg. If extrapolated on a mg/kg basis, this corresponds to a daily intake of 165–990 mg in a 70 kg human. Extrapolation by the 3/4 power standard gives human daily intakes of 21.2–127 mg (using mice) and 37.6–226 mg (using rats).

Recent studies in which whole *Spirulina* has been administered orally to rodents have also shown anti-inflammatory effects, in doses ranging from 150 to 1,000 mg/kg/day.[19–24] This amounts to intakes of 1–6.6 mg/kg/day PCB. Extrapolating on the basis of relative weight, this corresponds to an intake of 70–462 mg PCB in a 70 kg human. Extrapolating on the basis of the 3/4 power standard, it corresponds to an intake of 9–59 mg (mouse studies) or 16–106 mg (rat studies). The syndromes in which *Spirulina* demonstrated protective efficacy included adjuvant arthritis, MPTP-induced parkinsonism, doxorubicin-induced cardiomyopathy, and nephropathy mediated by cisplatin and cyclosporine; it is unlikely to be coincidental that activation of NADPH oxidase has been shown to be a key mediator of each of these syndromes.[33–44]

As noted, a heaping tablespoon of *Spirulina* contains approximately 100 mg of PCB. Thus, a regimen of 2 heaping tablespoons per day—arguably the highest intake that would be feasible on a long-term basis with well-motivated patients—would provide about 200 mg of PCB daily. This intake is thus within—and in some instances a bit beyond—the extrapolated dose ranges noted above. It should follow that—assuming that humans digest and metabolize *Spirulina*-bound PCB much like rodents do—a daily intake of 2 heaping tablespoons of *Spirulina* daily should have clinically useful antioxidant activity in humans. Thus, it would be reasonable to test such a regimen in the prevention or treatment of the wide range of clinical disorders in which overactivity of NADPH oxidase plays a pathogenic role. Assessing impact on clinical hypertension might be a good place to start, since an effective dose regimen could be expected to have a rapid and quantifiable impact.[45–51] In this regard, it is pertinent to note that, in a cohort of 50 middle-aged Czech subjects with Gilbert's syndrome (average age 50 years), only one was found to be hypertensive.[9] Intriguingly, Remirez *et al.*[20] stated that "a number of published reports suggest beneficial effects of this microalgae in hypertension . . ."—without, however, citing these reports.

Ultimately, the availability of PCB supplements—extracted from *Spirulina* or synthesized—should enable a broader assessment of the dose dependency of PCB's clinical benefits. Such supplements will improve the convenience of PCB ingestion, and make it possible to achieve PCB intakes greater than those feasible with whole *Spirulina*. Nonetheless, until PCB can be mass-produced inexpensively via chemical synthesis or bioengineered bacteria, ingestion of whole *Spirulina* will be the least expensive way to benefit from this phytonutrient. It should be noted that the relative absorption and bioefficacy of free PCB and *Spirulina*-bound PCB have not yet been assessed, in either rodents or humans; evidently, this issue requires attention.

Among health food devotees, *Spirulina* is frequently ingested in "smoothies"; a smoothie made by blending a cup of vanilla soy milk, one banana, and a heaping tablespoon of *Spirulina* is reasonably palatable (albeit it looks like frothy green slime!), and provides in addition soy isoflavones and an ample amount of potassium (about 1 g). Various fruit juices and whole fruits can also be used to produce *Spirulina* smoothies; for example, cinnamon-spiced apple juice works very well. The flavor of *Spirulina*, although unappealing to most people, is relatively mild, and thus susceptible to masking by a variety of flavors.

SAFETY CONSIDERATIONS AND ANCILLARY BENEFITS

With respect to safety considerations, it should be noted that *Spirulina* once was a staple of the Aztec diet, and rodents show no ill effects when fed diets in which *Spirulina* constitutes 30% of total weight.[52–54] Fifteen grams of *Spirulina* provides about 70 mg of total carotenoids—carotenes (54%) and xanthophylls (46%); this may make an ancillary contribution to the physiological antioxidant activity of this food. Of particular note is the zeaxanthin content—12 mg/15 g; if this has reasonable bioavailability, it could have a worthwhile impact on protective macular pigment.[55,56] The cell wall polysaccharide content of *Spirulina* may be a mediator of the immunomodulatory effects reported with *Spirulina* ingestion; *Spirulina* polysaccharides can activate antigen-presenting cells and monocytes via Toll-like receptor 2.[57–59] These polysaccharides ("spirulinan") are also reported to suppress the proliferation of vascular smooth muscle cells *in vitro*[60]; whether this effect is germane to orally ingested *Spirulina* is not clear. Sixty percent of the dry mass of *Spirulina* is protein; 2.3% of this protein is methionine, a fraction comparable to that in rice. Although the lipid content of spirulina is modest, this alga is unusually rich in gamma-linolenic acid—about 1.3% of dry weight; thus, an intake of 30 g of *Spirulina* daily would provide about 390 mg of gamma-linolenic acid, a dose that is, however, considerably lower than that shown to exert clinical anti-inflammatory activity in rheumatoid arthritis.[61] In one controlled but not double-blind study, 2 g of *Spirulina* daily was reported to improve glycemic control and serum lipids in type 2 diabetics[62]; it is not clear what mediates this effect (soluble fiber?), or whether this effect (if real) might be of a greater and more clinically significant magnitude with the higher doses of *Spirulina* recommended here.

It must be borne in mind, of course, that complete inhibition of NADPH oxidase would be inappropriate and dangerous, particularly in light of the key role which NADPH oxidase plays in phagocyte bactericidal mechanisms and in T cell regulation.[63,64] Fortunately, people with Gilbert's syndrome, characterized by chronic moderate (two- to threefold) up-regulation of free bilirubin levels, have not been noted to be especially prone to infection—even though they are at reduced risk for vascular disorders. This suggests that moderate, partial inhibition of NAPDH oxidase can be associated with worthwhile health benefits without entailing an important compromise of immune defenses—perhaps in part because phagocytes have recourse to ancillary bactericidal mechanisms.[65] In any case, once PCB supplements are available, their use could be temporarily discontinued when persistent or life-threatening infections develop. The impact of whole *Spirulina* on infection may be complex, since, as noted, the cell wall polysaccharides of *Spirulina* can act as a stimulant to macrophages.

NOTE ADDED IN PROOF

Since the submission of this review, Riss *et al*.[66] have reported that orally administered phycocyanin or whole *Spirulina* "powerfully prevents the development of atherosclerosis" in cholesterol-fed hamsters. Although this appears to be the first published evidence that *Spirulina* has vascular-protective potential, it is consistent with the central role that NADPH oxidase plays in atherogenesis.

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