Natural Product Therapeutics and COVID-19: The Case for Clinical Development of Spirulina Extracts

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https://www.algaesciences.com/post/natural-product-therapeutics-and-covid-19-the-case-for-clinical-development-of-spirulina-extracts

Abstract

In the light of the current paucity of therapeutic options for acute coronavirus 2019 (COVID-19), it is timely to consider broadening biopharmaceutical development options to include natural products. Extracts of Spirulina, a widely consumed blue-green cyanobacterium, demonstrate antiviral, immunomodulatory and anti-inflammatory properties in preclinical studies. These properties are attributable to the Spirulina phycobiliprotein-phycocyanin complex. Available preclinical data supports the potential application of phycocyanin-rich Spirulina extracts as disease modifying therapeutics in COVID-19, especially for lung injury and cytokine release syndrome.

Introduction

According to clinicaltrials.gov, there are over 1400 interventional trials underway for novel coronavirus 2019 (COVID-19). No natural product trials are currently listed, and the largest group of treatments is immunostimulant and anti-inflammatory category aiming to modify the course of the condition from early infection to Adult Respiratory Distress Syndrome (ARDS). With few exceptions, current clinical trials in COVID-19 test drugs that are already registered or currently in clinical development for other indications. Given the gravity of the pandemic, especially in terms of in-hospital mortality, there is an urgent need to explore new therapeutic approaches, especially if these potential therapies are safe ("primum non nocere") and where a robust scientific hypothesis and pre-clinical evidence is available. McCartey and Nicolantonio (2020) proposed that certain natural products and in particular Spirulina extracts have potential for boosting type 1 interferon response in RNA virus infection, and thus should be tested in COVID-19. The mechanism of action of Spirulina may be directly linked to the antioxidant activity of its chromophore, phycocyanobilin that is closely related to bilirubin and biliverdin intermediates of the human heme biosynthetic and salvage pathways (Manirafasha 2016). Phycocyanin is the most bioactive part of phycocyanobilin and is widely used as a natural blue colorant in foods and cosmetics.

Spirulina, a phycocyanin-containing cyanobacterium, has been used for millennia as a plant-based remedy and health food. Teas et al. (2004) hypothesized that regularly consumed seaweed and Spirulina may confer some protection against HIV infection and reduce viral load and Chen et al. (2016) demonstrated that Spirulina extract inhibits influenza virus replication and reduces virus-induced mortality. The anti-inflammatory, antiviral properties, and safety of Spirulina and phycocyanin-rich Spirulina extracts are well documented (Deniz 2017).

A role for Spirulina extracts in COVID-19 Cytokine Storm and ARDS

High critical care mortality in COVID-19 has been attributed to a combination of hyper-inflammatory syndromes, coagulation disorders and the pathological activation of currently unknown immunological pathways. Elevated serum concentrations of the cytokine interleukin-6 (IL-6) and other inflammatory cytokines are hallmarks of severe COVID-19 (Moore 2020). Cytokine Release Syndrome (CRS) is common in patients with COVID-19 and elevated serum IL-6 correlates with respiratory failure, ARDS, and adverse clinical outcomes. Elevated serum C-reactive protein (CRP), a protein whose expression is driven by IL-6, is also a biomarker of severe betacoronavirus infection (Moore 2020).

Yoshimoto et al. (2019) demonstrated that in a co-culture of a human colorectal cancer cell line (Caco-2) differentiated U937 macrophages in the presence of sodium butyrate (SB) and/or lipopolysaccharide (LPS), phycocyanin attenuated cell damage and suppressed IL-6 and IL-8 while enhancing TGF-β1 production. Phagocytic and bactericidal activities of the differentiated U937 cells were also regulated. Phycocyanin contributes to protecting against inflammation and to regulating macrophages in the mucosal immune response partly through release of cytokines in the presence of butyrate. In a rat study, Shih et al. (2009) demonstrated the anti-inflammatory activity of phycocyanin partly through the inhibition of pro-inflammatory cytokine formation, inducible nitric oxide synthase (iNOS) and cyclooxygeanase-2 (COX-2) expression. Treatment with (30 or 50 mg/kg, IP) phycocyanin significantly attenuated inflammatory nociception and the induction of iNOS and COX-2 and was accompanied by an inhibition of the formation of TNF-alpha, prostaglandin E2, nitrate and myeloperoxidase activity.

González et al. (1999) demonstrated that phycocyanin inhibited inflammatory cell infiltration and reduced cell injury in acetic acid induced colitis in rats. Phycocyanin (150, 200 and 300 mg/kg p.o.) was administered 30 min before induction of colitis with 1 ml of 4% acetic acid (by enema). Twenty-four hours later, myeloperoxidase (MPO) activity was determined and histopathological and ultrastructural studies were carried out on the colonic tissue. Phycocyanin substantially reduced MPO activity and inflammatory cell infiltration, thereby demonstrating antioxidant and free-radical scavenging properties. Prabakaran et al. (2020) found phycocyanin

had am anti-inflammatory effect such as inhibition of albumin denaturation, antiprotease, hypotonicity-induced hemolysis and anti-lipoxygenase activities have been recorded maximum level at 500 μg/mL.

Leung et al. (2013) found that phycocyanin inhibited the inflammatory response and apoptosis in injured rat lung tissue. Rats were challenged with lipopolysaccharide (LPS) (5 mg/kg body weight) intratracheally to induce ALI. After 3 hours, phycocyanin (50 mg/kg body weight, i.p.) was administered for 3 hours. Treatment with phycocyanin significantly inhibited LPS-induced protein concentration, nitrite/nitrate levels, release of proinflammatory cytokines, the number of total polymorphonuclear cells in bronchoalveolar lavage fluid, and lung edema and there was a remarkable improvement of lung histopathology. Furthermore, phycocyanin significantly attenuated LPS-induced myeloperoxidase activity, expression of inducible nitric oxide synthase, and cyclooxygenase-2 as well as nuclear factor-kappa B (NF-κB). Additionally, phycocyanin significantly downregulated proapoptotic proteins such as caspase-3 and Bax and upregulated antiapoptotic proteins such as Bcl-2 and Bcl-XL. These findings indicate that phycocyanin could be potentially useful for the treatment of ALI.

In the light of increasing evidence of multi-organ damage in COVID-19, Khan et al. (2006) found, in a model of rat myocardial injury, that phycocyanin improved the recovery of cardiac function by enhancing the activation of ERK1/2 and the expression of Bcl-2. This study also revealed a fourfold activation of Akt at 10 min of reperfusion. Recent data demonstrate the important role of the PI3K-Akt signaling pathway in survival of cardiomyocytes as well as in the protection against myocardial injury in mice and there is evidence of a possible role of phycocyanin in mediating these pathways. Phycocyanin has a role in the recovery of cardiac function following myocardial injury by enhancing the activation of ERK1/2 and the expression of Bcl-2 and also by attenuating the activation of p38 MAPK and caspase-3.

Spirulina extract – antiviral activity

There is evidence of the in-vitro viricidal and bactericidal effectiveness and infectivity attenuation properties of Spirulina extracts, mainly phycocyanin (Ramarkrishnan 2013). (McCartey and Nicolantonio 2020). Ayehunie et al. (1998) found that Spirulina extracts inhibited HIV-1 replication in human T-cell lines, peripheral blood mononuclear cells (PBMC), and Langerhans cells (LC). Extract concentrations ranging between 0.3 and 1.2 mcg/ml reduced viral production by approximately 50% and the IC50 of the extract for PBMC growth ranged between 0.8 and 3.1 mg/mL. The extract inactivated HIV-1 infectivity directly when pre-incubated with the virus before addition to human T-cell lines. Spirulina extract inhibited Herpes simplex virus type-1 (HSV-1) replication in HeLa cells interfering with the virus entry into host cells (Ramarkrishnan 2013). The calcium Spirulan (Ca-Sp) a sulfated polysaccharide isolated from Spirulina inhibits virus replication and exhibits antiviral activity against the HSV-1, influenza

virus, human cytomegalovirus, mumps virus, measles virus and human immunodeficiency virus type 1 (HIV-1) (Hayashi 1996). The in vitro replication of HIV-I is significantly inhibited by an aqueous Spirulina extract in human T-cell lines, peripheral blood mononuclear cells and Langerhans, blood mononuclear cells (Ayenunie 1998). The anti-HIV and anti-HSV-1 activity of Spirulina extracts may be mediated through viral yield reduction and these finding support therapeutic testing (Hayashi 1996). Purified allophycocyanin, a PCB from Spirulina inhibited enterovirus 71 activity and neutralized the cytopathic effects induced by enterovirus 71 and slowed viral RNA synthesis and activated apoptosis in both human rhabdomyosarcoma cells and African green monkey kidney cells (Shih 2003). Chen et al. (2016) demonstrated the Spirulina extract inhibits influenza virus replication and reduces virus-induced mortality. Results showed Spirulina extract inhibited viral plaque formation and reduced viral replication in cell cultures. Underlying mechanisms involved and found that the Spirulina extract disrupted the hemagglutination of viral particles to erythrocytes, thus inhibiting the infection process (Chen 2016).

Safety Profile of Spirulina extracts

Spirulina extract, rich in phycocyanin, is a generally-recognized-as-safe for human consumption (Federal Register 2013) food colorant. The US 2013 GRAS determination for phycocyanin notes that: *the NOEL (No Observable Effect Level) for phycocyanins for humans to be between 108,000 to 184,500 mg/p/d. Taking into account the available safety information, the estimated intake of phycocyanins from the petitioned use of the spirulina extract, and the large margin of safety between the cumulative EDI (Estimated Daily Intake) and the NOEL. We also evaluated the potential allergenicity of spirulina extract. We reviewed a comparison of the known amino acid sequences of phycocyanins with the sequences of known protein allergens and determined that there is a low probability that the phycocyanins are protein allergens. We conclude that spirulina phycocyanins present an insignificant allergy risk to consumers of the color additive. Our conclusion regarding the safety of the petitioned use of the color additive is strengthened by the fact that the phycocyanobilin chromophore (the part of the molecule responsible for the coloring effect of the additive) is similar to certain bile pigments that are excreted from the liver via the gall bladder into the intestines. Based on a literature search and review, none of the bile pigments has been reported to produce any toxic effect, except in diseases caused by their presence in the blood due to inborn error of metabolism or other cause* (Federal Register 2013).

Jensen et al. (2016) evaluated the anticoagulant and platelet activation activity of high dose phycocyanin. In a randomized, double blind, placebo-controlled study, 24 subjects (male and female) consumed 1g of phycocyanin per day. Besides showing safety in terms of anticoagulant activity and platelet activation status, the results indicated some anti-inflammatory activity (relief of chronic pain) and improved hepatic function. To determine the embryo-fetal toxicity of Spirulina, 4 animal studies have been conducted (Chamorro 1989, Chamorro 1990, Kapoor 1999, Salazar 1996). Chamorro et al. (1989) administered Spirulina to pregnant rats from days 1–14, 1–21 and 7–14 of gestation with increasing doses (0-30g/100g body weight). It was found that Spirulina consumption did not change the maternal and fetal weight. No teratogenicity was detected even at the highest dose and longest duration.

Spirulina supplementation at doses much higher than any anticipated for human consumption did not cause embryotoxic effects. Similar results were obtained with a similar study in pregnant rats (Chamorro 1990). In another rat study, the effects of Spirulina alone or in combination with other supplements on rat pregnancy was investigated (Kapoor 1999). Maximal maternal weight gain was associated with Spirulina and a wheat gluten diet whereas a wheat gluten diet alone resulted less weight gain. Intake of Spirulina significantly increased litter size whereas birth weights of pups were comparable to those from other groups. A study with pregnant rats to assess general reproductive performance showed that Spirulina feeding did not change the body weight of male and female rats, and there were no signs of toxicity and Spirulina was not associated with any adverse effects on reproductive performance including fertility, gestation and abnormal pups (Salazar 1996). Spirulina had no detectable adverse effects on rat reproductive performance, embryo and fetal development and growth (Chamorro 1996).

Conclusions

There is pre-clinical data showing that the phycocyanin-rich Spirulina extract has antiviral, anti-inflammatory and immunomodulatory activity. Phycocyanin has been shown to safe for human consumption and there are no apparent side effect and drug interaction issues. Natural phycocyanin-rich Spirulina extracts should be tested in COVID-19, especially in the mitigation of cytokine release syndrome.

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Conflict of Interest Disclosures: Erik Reynolds and Leonard Lerer are employees of Back of the Yards Algae Sciences.

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