

# **Spirulina and its Chromophore Phycocyanobilin have Potential for Alleviation of Asthma**

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## **Abstract**

Activation of various isoforms of NADPH oxidase contributes to the pathogenesis of asthma at multiple levels: promoting hypercontractility, hypertrophy, and proliferation of airway smooth muscle; enabling lung influx of eosinophils via VCAM-1; and mediating allergen-induced mast cell activation. Bilirubin, which functions physiologically within cells as a feedback inhibitor of NADPH oxidase complexes, has been shown to have a favorable impact on each of these phases of asthma pathogenesis. Moreover, an intriguing case report notes that severe chronic asthma was markedly ameliorated in a patient with hepatitis B as long as he was hyperbilirubinemic. The spirulina chromophore phycocyanobilin (PhyCB), a homolog of bilirubin's precursor biliverdin, has recently been shown to mimic the inhibitory impact of biliverdin/bilirubin on NADPH oxidase activity, and spirulina's versatile and profound anti-inflammatory activity in rodent studies suggests that PhyCB may have potential as a clinical inhibitor of NADPH oxidase. These considerations suggest that spirulina or PhyCB-enriched spirulina extracts merit clinical evaluation in asthma.

## **Multiple Roles for NADPH Oxidase in the Pathogenesis of Asthma**

Accumulating research indicates that activation of NADPH oxidase complexes contributes in a number of complementary ways to the pathogenesis of asthma:

***Airway Smooth Muscle Hypercontractility*** – Airway smooth muscle (ASM) obtained by biopsy from patients with asthma is hypersensitive to agonist-induced contraction.<sup>1</sup> This ASM also shows signs of increased oxidative stress, including increased DNA oxidation. Nox4 is overexpressed in this ASM, and knockdown of Nox4 expression with small interfering RNA alleviates the hypercontractility of these cells. Studies with guinea pig ASM cells exposed to TNF-alpha – thought to be a key mediator of ASM hypercontractility in asthmatics – likewise demonstrate that activation of NADPH oxidase is necessary for induction of increased contractility, and that increased phosphorylation of myosin light chain (MLC) is a downstream mediator of this effect.<sup>2</sup> In vascular smooth muscle, oxidants promote MLC phosphorylation via activation of RhoA and Rho kinase, which in turn induce an inhibitory phosphorylation on the regulatory subunit of the myosin light chain phosphatase;<sup>3, 4</sup> arguably, this mechanism may also account for oxidant-mediated phosphorylation of MLC in ASM.

***Airway Smooth Muscle Hypertrophy and Hyperplasia*** – The hypertrophic and proliferative response of ASM cells in culture to serum or agonists such as TGF- $\beta$ 1 appears to likewise be mediated by Nox4 activation, as silencing of Nox4 or other measures known to inhibit NADPH oxidase activity render ASM more quiescent.<sup>5-7</sup> Pertinent downstream targets activated by Nox4-induced oxidants include NF-kappaB, ERK1/2, and mTORC1. Expansion of airway smooth muscle mass is a common feature of chronic asthma, leading to persistent smooth airway obstruction not reversible with bronchodilators.

***Eosinophil Influx*** – Pulmonary eosinophilia is a typical feature of asthma, and is suspected to exacerbate the syndrome by release of various pro-inflammatory mediators.<sup>8</sup> Circulating eosinophils access the lung parenchyma via VCAM-1 receptors on lung endothelial cells.<sup>9, 10</sup> (Lymphocytes also employ this transit mechanism, although they are less obligately dependent upon it.) Engagement of these receptors by eosinophils induces activation of Nox2-dependent NADPH oxidase activity in endothelium, and the resulting oxidative stress plays an obligate role in enabling infiltration of eosinophils into the lung.<sup>11, 12</sup> Hence, in Nox2-knockout mice rendered chimeric by irradiation and implantation of wild-type hematopoietic cells, lung eosinophil influx following intranasal challenge with ovalbumin (in mice previously sensitized to this protein) is substantially blunted in contrast to wild-type mice; moreover, the airway hyperresponsiveness following ovalbumin challenge is likewise blunted in these chimeric mice, consistent with a role for eosinophil influx in airway obstruction.<sup>13</sup>

***Mast Cell Activation*** – Agonists which promote mast cell degranulation, and boost mast cell production of Th2 cytokines such as IL-4 and IL-13, do so via a signaling pathway obligately dependent on NADPH oxidase activation; the Nox1 isoform appears to mediate this effect.<sup>14-16</sup> Mast cell activation evidently plays a crucial role in allergic asthma.

### **Strategies for Controlling NADPH Oxidase Activity**

These considerations suggest that therapeutic strategies capable of safely down-regulating NADPH oxidase activation in the lung could have major potential for controlling asthma. Indeed, systemic administration of the NADPH oxidase-inhibitory agent apocynin notably blunts airway hyperresponsiveness on lung inflammation in sensitized mice challenged with ovalbumin.<sup>17</sup> In mild human asthmatics, inhalation of aerosolized apocynin prior to and during exposure to ozone blunted the subsequent airway hyperresponsiveness in response to methacholine.<sup>18</sup>

Moreover, recent research has established that the profound physiological antioxidant activity of bilirubin – manifest intracellularly at low nanomolar concentrations – reflects inhibition of NADPH oxidase complexes; the mechanism and isoform specificity of this effect requires further clarification.<sup>19-22</sup> When cells are oxidatively stressed – oftentimes by overactivation of NADPH oxidase - induction of heme oxygenase-1 results in breakdown of heme, yielding carbon monoxide and biliverdin; the latter is quickly reduced to bilirubin, which provides feedback inhibition of NADPH oxidase.<sup>22</sup> Ohrui and colleagues have reported an intriguing case history in which a teenager with chronic hard-to-control asthma was hospitalized for acute hepatitis B.<sup>23</sup> The patient's serum bilirubin level tripled during the course of his hospital stay, and his physicians were intrigued to note that his intractable asthma almost completely remitted during this time, such that asthma medications could be discontinued. However, within a couple of weeks, his bilirubin levels normalized – and this was associated with return of his asthma. His physicians cleverly suggested that the antioxidant activity of bilirubin may have been responsible for his temporary remission.

Experimental studies likewise suggest that bilirubin may function physiologically to quell the asthma syndrome. In vitro, bilirubin has been shown to impede VCAM-1-dependent transendothelial migration; in a murine asthma model, i.p. administration of bilirubin was found to suppress lung infiltration by eosinophils and lymphocytes.<sup>24</sup> The ability of heme oxygenase-1 induction to antagonize ASM cell hypercontractility and proliferation has been traced to the bilirubin generated by this enzyme.<sup>25, 26</sup> Analogously, exposure of mast cells to low micromolar concentrations of bilirubin opposes agonist-

induced degranulation and up-regulation of adhesion, mimicking the impact of heme oxygenase-1 induction in this regard.<sup>27, 28</sup> Hence, bilirubin has been shown to antagonize each of the NADPH oxidase-dependent phases of asthma pathogenesis highlighted above.

### **Phycocyanobilin as a Clinically Feasible NADPH Oxidase Inhibitor**

Unfortunately, bilirubin is unsuitable for oral administration owing to its marked insolubility; its more soluble precursor biliverdin is more feasible in this respect, but there are no known rich sources of this chemical, and it is expensive to synthesize. It is therefore quite propitious that cyanobacteria such as spirulina are very rich sources – about 0.6% by dry weight – of the chromophore phycocyanobilin (PhyCB), a biliverdin metabolite.<sup>29</sup> Within cells, PhyCB is quickly converted to phycocyanorubin, whose structure is quite similar to that of bilirubin.<sup>30</sup> Indeed, PhyCB, likely via its metabolite phycocyanorubin, has been shown to inhibit NADPH oxidase complexes *in vitro* and *in vivo* with a dose-dependent potency similar to that observed with biliverdin/bilirubin.<sup>29, 31</sup> This phenomenon appears to explain why oral administration of spirulina or of phycocyanin (the spirulina protein which contains PhyCB as a covalently attached chromophore) has exerted profound anti-inflammatory effects in a host of rodent models of inflammation.<sup>29, 32</sup> More recently, oral phycocyanin has shown marked anti-atherosclerotic activity in cholesterol-fed hamsters, and nearly completely prevented nephrosclerosis in diabetic mice – syndromes known to be driven, in part, by NADPH oxidase activation.<sup>31, 33</sup>

In light of the foregoing discussion, it seems quite reasonable to propose that an adequate intake of spirulina, phycocyanin, or PhyCB-enriched spirulina extracts may have important clinical utility in asthma. Of course, this hinges on the presumption that humans can absorb and metabolize PhyCB much like rodents do – a proposal which still requires clinical confirmation. Arguably, assessing the clinical impact of high-dose spirulina on asthma could be a quick and highly feasible way of confirming that PhyCB has the antioxidant/anti-inflammatory potential in humans that it clearly does in rodents. Extrapolating from the doses that have proved highly effective in rodent models, it has been estimated that humans might need to take 15-30 g spirulina daily – or the equivalent intake of PhyCB – to achieve optimal anti-inflammatory effects.<sup>29</sup>

Moreover, if PhyCB can function as a clinically feasible NADPH oxidase inhibitor in humans, there is reason to suspect that it may have much broader utility for lung protection. A recent massive prospective epidemiological analysis in Britain found that people with relatively high serum bilirubin levels were at notably lower risk both lung cancer and chronic obstructive pulmonary diseases – an effect which arguably could reflect down-regulated activity of NADPH oxidase complexes in lung tissue.<sup>34</sup> Intravenous bilirubin infusion protects rats from bleomycin-induced pulmonary fibrosis, likely reflected the role of NADPH oxidase in TGF-beta signaling.<sup>35</sup> The same clinical group which noted improvement of asthma coincident with temporary hyperbilirubinemia, reported resolution of idiopathic pulmonary fibrosis in a patient who developed sustained elevated bilirubin owing to biliary tract obstruction.<sup>36</sup> The hypercontractility and hyperplasia of pulmonary vascular smooth muscle triggered by chronic hypoxia during the onset of pulmonary hypertension, appears to be mediated by oxidative stress in this vascular muscle that stems from both mitochondria and NADPH oxidase.<sup>4, 37-39</sup> The overexuberant lung inflammation that mediates death in “killer” influenzas appears to reflect viral activation of NADPH oxidase in lung epithelium.<sup>40-42</sup> Endotoxin-induced acute lung injury in rats – a model for acute respiratory distress syndrome – is substantially blunted by biliverdin administration; mortality is also

markedly decreased.<sup>43</sup> NADPH oxidase activation seems likely to play a pathogenic role in cystic fibrosis.<sup>44, 45</sup> It can be concluded that, if spirulina/PhyCB do indeed have useful clinical activity in asthma, they may have a much broader potential for promotion of pulmonary health.

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