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REVIEW

Review – Nutraceuticals Can Target Asthmatic Bronchoconstriction: NADPH Oxidase-Dependent Oxidative Stress, RhoA and Calcium Dynamics

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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. Free 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. The spirulina chromophore phycocyanobilin (PhyCB), a homolog of bilirubin's precursor biliverdin, can 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. Hence, spirulina or PhyCB-enriched spirulina extracts merit clinical evaluation in asthma. Promoting biosynthesis of glutathione and increasing the expression and activity of various antioxidant enzymes – as by supplementing with N-acetylcysteine, Phase 2 inducers (eg, lipoic acid), selenium, and zinc – may also blunt the contribution of oxidative stress to asthma pathogenesis. Nitric oxide (NO) and hydrogen sulfide (H_2S) work in various ways to oppose pathogenic mechanisms in asthma; supplemental citrulline and high-dose folate may aid NO synthesis, high-dose biotin may mimic and possibly potentiate NO's activating impact on soluble guanylate cyclase, and NAC and taurine may boost H_2S synthesis. The amino acid glycine has a hyperpolarizing effect on airway smooth muscle that is bronchodilatory. Insuring optimal intracellular levels of magnesium may modestly blunt the stimulatory impact of intracellular free calcium on bronchoconstriction. Nutraceutical regimens or functional foods incorporating at least several of these agents may have utility as nutraceutical adjuvants to standard clinical management of asthma.

Keywords: asthma, bronchoconstriction, calcium, NADPH oxidase, RhoA, oxidative stress

Introduction

Asthma is a chronic inflammation of the airways of the lungs, characterized by reversible airflow obstruction, variable and recurring symptoms, and allergen triggered bronchospasms.[1] Genetic and environmental factors are predisposing factors and wheezing, shortness of breath, coughing and chest tightness are frequent presenting symptoms. Asthma is thought to afflict about 300 million people worldwide, and its prevalence over the last several decades has increased markedly.[2] There is no

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definitive cure for the disease. Symptoms can be prevented by avoiding allergens and irritants, or treated by inhaled corticosteroids, long-acting beta agonists or anti-leukotriene agents.[3] Various diets and food supplements have been suggested for aiding asthma control, but a comprehensive review describing how nutraceuticals might target asthmatic bronchoconstriction is lacking.[4] This review summarizes what is known regarding the molecular biology underlying the pathogenesis of asthma, focusing in particular on the role played by reactive oxygen species generated by NADPH oxidase complexes, and proposes that certain specific nutraceuticals have potential for asthma control, meriting clinical evaluation in this regard.

Pathogenesis of Allergic Asthma

Allergic asthma constitutes an inflammatory stew in which Th2 lymphocytes, mast cells, basophils, and eosinophils, in response to an allergen challenge, congregate in the lung and produce a range of autacoids and toxins that increase resistance to air flow both by inducing bronchoconstriction and by increasing mucus production.[5] In the longer term, bronchial hyperplasia can evolve, further increasing airway resistance in a manner not susceptible to acute therapeutic control.[6]

Mechanisms of Bronchoconstriction in Asthma

Figure 1 provides an overview of the molecular biology underlying asthmatic bronchoconstriction. Autacoids such as histamine, prostaglandins and leukotrienes act via G protein-coupled receptors to induce activation of the G protein RhoA and phospholipase C, while exerting a depolarizing effect on smooth muscle plasma membrane that promotes calcium influx via voltage-gated calcium channels.[7] RhoA activity is boosted by $G_{12/13}$-mediated activation of RhoA GEFs (guanosine nucleotide exchange factors) that induce RhoA binding to GTP.[7,8] Activated RhoA then stimulates Rho-activated kinase (ROCK), which confers an inhibitory phosphorylation on the myosin light chain phosphatase (MLCP) complex.[7] Concurrently, this complex is also inhibited by the protein CPI-17; the inhibitory efficacy of this protein is activated by a phosphorylation conferred by protein kinase C (PKC), which in turn is activated by G protein-mediated stimulation of phospholipase C activity (PLC).[9] Meanwhile, broncho-constrictive autacoids boost the activity of myosin light chain kinase by inducing calcium influx via L-type voltage-sensitive calcium channels; these autacoids promote a depolarization of the plasma membrane that induces this influx. With respect to histamine, stimulation of H1 receptors activates PKC, which in turn confers an inhibitory phosphorylation on Kv7.5 potassium channels, resulting in depolarization and consequent calcium influx.[10,11] The net impact of these mechanisms is to boost the activity of MLCK while suppressing that of MLCP, amplifying the Ser-19 phosphorylation of the 20 kDa myosin light chain that induces smooth muscle constriction.[12,13]

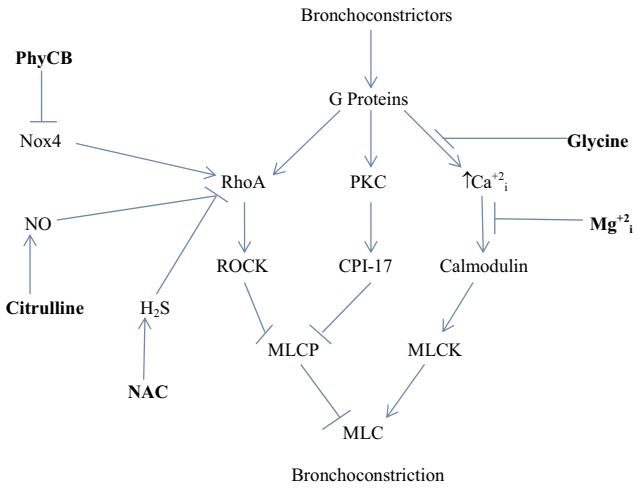

Figure 1 Regulation of bronchial constriction. Nutraceuticals with potential for intervening in this process are highlighted in bold. i–intracellular.

The bronchial smooth muscle of asthmatics typically displays increased expressions of the Nox4 isoform of NADPH oxidase; moreover, the hypersensitivity of asthmatic bronchial smooth muscle to bronchoconstrictors is ameliorated by inhibition of Nox4.[14] This may reflect an oxidant-induced amplification of RhoA expression and activity. Oxidant production by Nox4 has been shown to increase RhoA/ROCK expression in vascular smooth muscle, and other studies show that stimulation of oxidant production in smooth muscle boosts RhoA activity or expression.[15–18] One possible explanation for this phenomenon is that oxidants suppress the expression of MiR-133a, which targets the 3′ untranslated region of RhoA mRNA, promoting its degradation; hence, oxidants would be expected to up-regulate RhoA mRNA.[18] The Th2-generated cytokines IL-4 and IL-13 can also increase RhoA expression in bronchial smooth muscle.[19] On the other hand, activation of RhoA is opposed by the bioactivities of both nitric oxide (NO) and hydrogen sulfide (H_2S). NO-mediated activation of soluble guanylate cyclase (sGC) stimulates synthesis of cyclic GMP (cGMP),

which in turn binds to and activates protein kinase G (PKG). Activated PKG can then confer a phosphorylation on RhoA that prevents it from interacting with its GEF and binding GTP.[20,21] H_2S can interact directly with RhoA via reversible S-sulfhydration of a cysteine group; this likewise prevents the activation of RhoA.[22,23] Concurrently, H_2S acts to upregulate NO bioactivity, by reversing a peroxynitrite-mediated oxidation of sGC that renders it non-responsive to NO.[24] H_2S can also boost cellular cGMP levels via inhibition of phosphodiesterase-5, which degrades cGMP.[25,26] Hence, measures which inhibit Nox4 activity, amplify or mimic NO bioactivity, or boost H_2S synthesis can be expected to oppose bronchoconstriction by decreasing the expression or activation of RhoA.

Multiple Roles for NADPH Oxidase in the Pathogenesis of Asthma

The foregoing discussion cites evidence that Nox4 overexpression in bronchial smooth muscle is a key mediator of bronchoconstriction in asthma. However, NADPH oxidase activity plays a number of additional key roles in the pathogenesis of asthma:

Airway Smooth Muscle Hypertrophy and Hyperplasia

The hypertrophic and proliferative response of airway smooth muscle (ASM) cells in culture to serum or agonists such as TGF-β1 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.[27–29] 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.

Ciliary Dysfunction

In airway epithelium, reduced cilia beat frequency is observed in patients with a neutrophilic subtype of asthma; this impairs mucus clearance and is linked to increased risk for lung infections.[30,31] Studies with ex vivo epithelial strips from such patients indicate that Nox4 is overexpressed in this epithelium, and that reduction in beat frequency is reversed by a chemical that inhibits specifically the Nox1 and Nox4 isoforms of NADPH oxidase.[31]

Eosinophil Influx

Pulmonary eosinophilia is a typical feature of asthma, and is suspected to exacerbate the syndrome by release of various pro-inflammatory mediators.[32] Circulating eosinophils access the lung parenchyma via VCAM-1 receptors on lung endothelial cells.[33,34] (Lymphocytes also employ this transit mechanism, although they are less obligately dependent upon it.) Engagement of endothelial VCAM-1 receptors by eosinophils induces activation of Nox2-dependent NADPH oxidase activity in endothelium, and the resulting oxidant production plays an obligate role in enabling infiltration of eosinophils into the lung.[35,36] 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.[37]

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.[38–40] Mast cell activation evidently plays a crucial role in allergic asthma.

Evidently, agents which can safely down-regulate NADPH oxidase activity, or which counteract the signaling impact of oxidants by either promoting catabolism of oxidants, or by acting to reverse the oxidation of acidic cysteine groups induced by hydrogen peroxide, should have interesting potential for preventing or controlling asthma. Figure 2 depicts the multiple roles of NADPH oxidase-generated oxidants in the pathogenesis of asthma.

Nutraceutical 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 and lung inflammation in sensitized mice challenged with ovalbumin.[41] In mild human

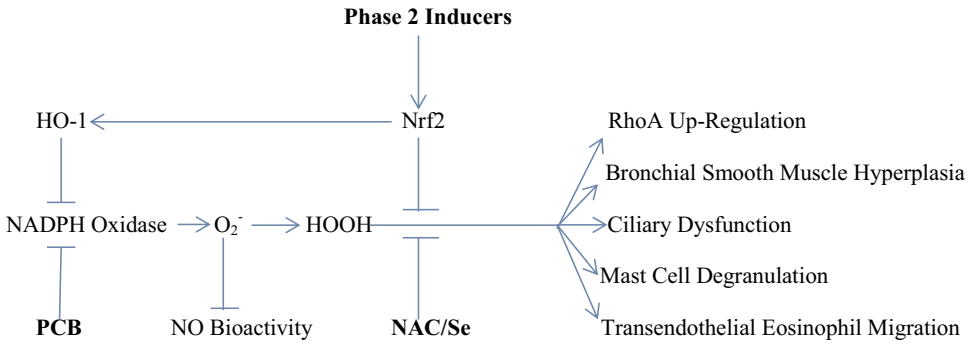

Figure 2 The multiple roles of NADPH oxidase-driven oxidative stress in the pathogenesis of asthma. Nutraceuticals with potential for intervening in this process are highlighted in bold.

asthmatics, inhalation of aerosolized apocynin prior to and during exposure to ozone blunted the subsequent airway hyperresponsiveness in response to methacholine.[42]

Moreover, recent research has established that the profound physiological antioxidant activity of bilirubin – observed intracellularly at low nanomolar concentrations – reflects inhibition of NADPH oxidase complexes; the mechanism and isoform specificity of this effect requires further clarification.[43–46] 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.[46] 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.[47] 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 insightfully 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 a mouse model of allergic asthma engendered by repeated nasal administration of aspergillus/ovalbumin proteins, i.p or i.v. administration of bilirubin nanoparticles markedly reduced airway hyperresponsiveness to methacholine, decreased cell count in bronchoalveolar fluid (BALF), and suppressed eosinophil influx.[48] In vitro, bilirubin has been shown to impede VCAM-1-dependent trans endothelial migration; in a murine asthma model, i.p. administration of bilirubin was found to suppress lung infiltration by eosinophils and lymphocytes.[49] The ability of heme oxygenase-1 induction to antagonize ASM cell hypercontractility and proliferation has been traced to the bilirubin generated by this enzyme.[50,51] 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.[52,53] Hence, bilirubin has been shown to antagonize most 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.[54] Within cells, PhyCB is quickly converted to phycocyanorubin, whose structure is quite similar to that of bilirubin.[55] 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.[54,56] This phenomenon likely explains 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.[54,57] 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.[56,58]

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.[54] However, a small non-blinded controlled trial in adult asthmatics found that one gram daily of spirulina provided clinical benefit comparable to standard medication, and that the combination of spirulina with medication produced the best clinical outcomes.[59] A comparable study in a larger group with a higher dose of spirulina would be warranted.

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 the UK found that people with relatively high serum bilirubin levels were at notably lower risk for both lung cancer and chronic obstructive pulmonary diseases – an effect which arguably could reflect down-regulated activity of NADPH oxidase complexes in lung tissue.[60] Intravenous bilirubin infusion protects rats from bleomycin-induced pulmonary fibrosis, likely reflected the role of NADPH oxidase in TGF-beta signaling.[61] The same clinical group which noted improvement of asthma in a patient during an episode of temporary hyperbilirubinemia, also reported resolution of idiopathic pulmonary fibrosis in a patient who developed sustained elevated bilirubin owing to biliary tract obstruction.[62] 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.[17,63–65] The overexuberant lung inflammation that mediates death in "killer" influenzas appears to reflect viral activation of NADPH oxidase in lung epithelium.[66–68] 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.[69] NADPH oxidase activation seems likely to play a pathogenic role in cystic fibrosis.[70,71] 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.

There is also some evidence that spirulina, and perhaps PhyCB, has the potential to down-regulate the induction of Th2 cells that play a central role in the pathogenesis of asthma and allergic rhinitis. In a double-blind trial, administration of 2 g spirulina daily was found to lower the ex vivo production of phytohaemaglutinin-stimulated peripheral blood mononuclear cells by a significant 32%.[72] Such an effect could be expected to diminish the differentiation of Th2 cells. This is paralleled by evidence that i.p. administration of bilirubin can reduce BLF content of the Th2 cytokines IL-4, IL-5, and IL-13; moreover, in vitro, bilirubin nanoparticles dose-dependently reduce the induction of IL-4-producing T cells in stimulated CD4+ lymphocytes.[48] Curiously, there is also evidence that bilirubin/biliverdin and PhyCB may modulate T cell development by promoting induction of Treg cells.[73–75] This latter effect might hinge on the ability of bilirubin (and possibly PhyCB's metabolite phycocyanorubin?) to act as an agonist for the arylhydrocarbon receptor, which likewise promotes Treg induction.[76–78]

Increasing Lung Glutathione Levels May Counter Some Pathogenic Effects of Oxidative Stress

Many of the pro-inflammatory effects of oxidative stress – including presumably some of those stemming from Nox4 activity in asthma – are mediated by hydrogen peroxide, which oxidizes acidic cysteine groups in signaling proteins or enzymes to sulfenic acid.[79,80] Reduced glutathione, working in concert with glutaredoxin, can reverse these oxidations, restoring protein-bound cysteine to its native form.[81–83] In this way, glutathione works to counteract oxidant-induced pro-inflammatory signaling. The availability of cysteine is rate-limiting for glutathione synthesis, and many studies – both in rodents and humans – demonstrate that supplementation with N-acetylcysteine (NAC)

can increase tissue glutathione levels;[84,85] NAC is better tolerated and more stable than free cysteine when administered orally, and readily gives rise to free cysteine once absorbed. Hence, there is reason to suspect that supplemental NAC could aid control of asthma. Indeed, two groups have reported favorable effects of NAC administration in ovalbumin-induced asthma in rodents.[86–88] In a controlled clinical study, enrolling individuals hyperresponsive to methacholine challenge, 6 days of NAC pretreatment (600 mg three time daily) reduced baseline methacholine responsiveness by about 20%, and also blunted the ability of diesel exhaust to increase airway hyperresponsiveness.[89] However, a controlled trial of NAC (600 mg twice daily) in patients experiencing asthma exacerbations failed to observe clinical benefit.[90]

Glutathione synthesis can also be promoted by administration of so-called phase 2 inducers, which stimulate activity of the nrf2 transcription factor to increase expression of a number of antioxidant enzymes, including γ-glutamylcysteine synthetase, rate-limiting for glutathione synthesis.[91] Enzymes whose expression is enhanced by phase 2 inducers work in conjunction with glutathione and the small proteins thioredoxin and glutaredoxin to catabolize oxidants such as hydrogen peroxide, or to reverse the oxidizing impact of hydrogen peroxide on protein sulfhydryl groups. The most clinically developed of phase 2 inducers is the natural cofactor lipoic acid (LA), which has been shown to be therapeutically beneficial in diabetic neuropathy in doses of 600 mg 2–3 times daily.[92,93] LA administration has been evaluated in ovalbumin-induced asthma in mice; LA decreased airway hyperresponsiveness, eosinophil influx, and markers of oxidative stress in bronchoalveolar lavage fluid.[94] In a longer term study in this model, LA administration suppressed airway remodeling.[95] The natural phase 2 inducer ferulic acid, used in China as a medication for cardiovascular disorders, likewise has been found to ameliorate ovalbumin-induced asthma in mice.[96,97]

Phase 2 inducers have the additional merit of boosting expression of heme oxygenase-1, which degrades heme to generate free bilirubin within cells.[98] This effect could evidently complement the utility of PhyCB for suppressing NADPH oxidase activity within the lung.

The essential mineral selenium is an obligate component of certain phase 2-inducible antioxidant enzymes, including thioredoxin reductase and various isoforms of glutathione reductase.[99] Hence, it is reasonable to presume that achieving adequate selenium status in asthma patients with poor baseline selenium nutrition may favorably impact asthma control. Indeed, a recent meta-analysis has concluded that patients with asthma tend to have lower plasma selenium levels than controls.[100]

NO and H_2S Bioactivity Both Oppose Pathogenic Mechanisms in Asthma

In the healthy lung, nitric oxide (NO), produced primarily by airway epithelium, vascular endothelium, and neurons via the constitutive endothelial and neuronal forms of NO synthase, acts directly on bronchial smooth muscle to promote bronchodilation and oppose hypertrophy and hyperplasia.[101–104] NO produced by airway epithelium boosts ciliary beat frequency.[105,106] NO production by vascular endothelium opposes the influx of eosinophils into lung parenchyma.[107–110] The effects of NO bioactivity on bronchial smooth muscle, epithelial ciliary function, trans endothelial eosinophil influx, and mast cell activity are in opposition to those of lung oxidative stress, which is not entirely coincidental, as superoxide and its downstream products act in various ways to oppose NO bioactivity. Superoxide reacts avidly and spontaneously with NO to produce the potent oxidant peroxynitrite; peroxynitrite, in turn, can cause an inhibitory oxidation of sGC, and also cause uncoupling of NO synthase via oxidation of its obligate cofactor tetrahydrobiopterin.[111,112] Oxidative stress can also impair the activity of dimethylarginine dimethylaminohydrolase (DDAH), the enzyme which catabolizes the natural metabolite asymmetric dimethylarginine (ADMA).[113] The latter also uncouples NO synthase, so a deficit in DDAH activity tends to promote this uncoupling by boosting tissue levels of ADMA.[114] Conversely, genetic overexpression of DDAH1 in mouse models of asthma attenuates lung inflammation, presumably by enhancing coupled NO synthase activity.[115]

Theoretically, nutraceutical measures which boost NO synthase activity should be helpful in asthma control. However, in seeming paradox, exhalation of NO in asthma patients tends to be elevated, reflecting the ability of proinflammatory cytokines in the lungs to boost expression of the inducible form of NO synthase (iNOS).[116–118] Indeed, higher levels of exhalate NO tend to correlate with severe disease in asthma patients, associated with greater inflammation within the lungs.[114,119] The failure of this elevated NO production to confer notable benefit in asthma patients appears to reflect impaired activity of sGC. This

impairment is attributable, in part, to oxidative inactivation of sGC, likely mediated by peroxynitrite.[111] Additionally, the expression of sGC, both at the mRNA and protein level, is reduced in the ASM of mice with ovalbumin-induced asthma; why this occurs remains mysterious.[120,121]

Measures which promote proper coupling of NO synthase should be doubly beneficial for asthma control, as these could be expected both to enhance NO production and bioactivity, and to decrease production of superoxide.[114] Blood and lung levels of ADMA tend to be elevated in asthma patients; indeed, elevation of ADMA may be a key reason why obesity tends to increase asthma severity.[115,122–126] Moreover, increases in lung arginase expression in asthma diminish the arginine/ADMA ratio, further promoting uncoupling of NO synthase. Nutritional elevation of plasma and tissue arginine levels – most effectively achieved by supplementation with the arginine precursor citrulline[127,128] – promotes recoupling of NO synthase in the context of elevated ADMA and arginase.[114,129] (Indeed, citrulline functions as a competitive inhibitor of arginase.[130]) Hence, citrulline supplementation may exert an antioxidant effect on the lungs of asthmatics, while concurrently boosting NO production. Arginine supplementation has provided benefit in several rodent models of asthma – albeit not all.[131–135] Conversely, elevation of plasma ADMA via continuous subcutaneous infusion potentiates ovalbumin-induced allergic lung inflammation in mice, whereas DDAH1 overexpression is protective in this regard.[136] In a recent open clinical study, obese asthmatics with NO exhalation in the low-normal range were treated with 15 g citrulline daily for a minimum of 14 days; forced vital capacity and an index of quality of control improved slightly but significantly, exhalation of NO increased, and the plasma ratio of arginine to ADMA nearly doubled.[137]

With respect to the possibility that increased ADMA is a mediator of the greater severity of asthma in diabetics, it is of interest that treatment with the drug metformin, which has been shown to lower ADMA levels in diabetics, has been associated epidemiologically with lower risk for asthma in diabetics, and better control of asthma in diabetics who already have it.[138–140] Hence, metformin may be a good therapy choice in asthmatic diabetics. Berberine, an herbally-derived nutraceutical used commonly for diabetes treatment in China, and which replicates metformin's ability to activate AMP-activated kinase, has been shown to favorably influence ovalbumin-induced asthma in rats.[141]

It seems likely that peroxynitrite-mediated oxidation of tetrahydrobiopterin also contributes to uncoupling of lung NO synthase in asthma.[112] When the vascular system is under oxidative stress, administration of high-dose folate (eg, 10–80 mg daily) helps to recouple eNOS.[142–145] This seems to reflect two effects. Reduced metabolites of folate produced within cells are effective scavengers of peroxynitrite-derived radicals (an effect that might conceivably protect sGC activity as well).[146] Moreover, high levels of folate induce increased expression of dihydrofolate reductase in endothelial cells; this enzyme functions to reduce dihydrobiopterin to tetrahydrobiopterin, reversing the oxidizing impact of peroxynitrite on this cofactor.[147–150] Whether high-dose folate can exert a comparable inductive effect in lung epithelial cells merits study.

Metallothionein (MT) can function to quench peroxynitrite-derived oxidants, and ovalbumin-induced asthma is more intense in MT-knockout mice.[151–153] Conversely, zinc supplementation can boost MT expression, which may help to rationalize the favorable impact of zinc supplementation on asthma in pilot clinical trials and in mouse models.[154–159] A meta-analysis has determined that plasma zinc levels tend to be lower in asthma patients than controls.[100]

Another way to protect sGC from oxidative inhibition is to boost production of hydrogen sulfide (H_2S). This gaseous mediator works in a variety of ways to complement the bioactivity of NO.[160] In particular, H_2S, much more effectively than glutathione, can reactivate oxidized sGC by re-reducing it.[24] Moreover, in cells prominently expressing phosphodiesterase 5 (PDE5), it can up-regulate cGMP levels by inhibiting this enzyme (like the pharmaceutical PDE5 inhibitors used to treat erectile dysfunction).[161]

The primary enzymes which produce H_2S, using cysteine as a substrate, are cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE); these are expressed in airway smooth muscle, as well as the endothelium and smooth muscle of the pulmonary vasculature.[162] Serum levels of H_2S are depressed in asthma patients – more so in those with disease exacerbations as opposed to stable asthma.[163] In patients with acute asthma, serum H_2S correlates directly with forced expiratory volume in one second (FEV_1). In mice with ovalbumin-induced asthma, both serum and lung H_2S are decreased, as is expression of CBS and CSE in lung

tissue.[164] Exogenous H_2S (provided via injection of NaHS) improved peak expiratory flow and alleviated lung inflammation in this syndrome – whereas inhibition of CSE with the drug D,L-propylarginine exacerbated it.[164]

H_2S can work in a range of ways to ameliorate asthma, and some of these are independent of its amplifying impact on NO bioactivity.[162] H_2S induces relaxation in tracheal smooth muscle by reducing calcium influx – an effect independent of NO.[165] It also acts in vitro to slow proliferation of ASM and airway fibroblasts, and to stabilize mast cells.[166–168] $NaSH_2$ administration decreases elevations of eotaxin in the lungs of mice with ovalbumin-induced asthma, suggesting that H_2S can slow influx of eosinophils.[169] Hence, its effects on the asthma syndrome appear to be comparable to those of NO.

Supporting Endogenous Production of H_2S

Although drugs achieving slow systemic release of H_2S are being developed, it may be feasible to boost endogenous H_2S production with nutraceuticals. Since cysteine availability is not saturating for either CBS or CSE, boosting lung cysteine levels with supplemental NAC can be expected to enhance lung H_2S.[170] Hence, NAC supplementation in asthma may serve a dual purpose, enhancing synthesis of both glutathione and H_2S.

With respect to the expressions of CBS and CSE, an intriguing recent study has shown that taurine supplementation increases expression of these enzymes in the aorta of mice; moreover taurine dose-dependently increases their expression in human mesenteric arteries ex vivo.[171] In human hypertensives, oral administration of 1.6 g taurine daily doubled serum levels of H_2S, while achieving reductions in both systolic and diastolic blood pressure that were significant with respect to placebo-treated patients.[171] It is credible to speculate that H_2S may be a primary mediator of the anti-atherosclerotic and anti-hypertensive effects of taurine extensively documented in rodent studies, as H_2S is known to work in multiple complementary ways to protect vascular health.[170,172,173] Whether taurine can influence CBS and CSE expression in lung has not yet been determined. Only a single study has evaluated taurine in a rodent asthma model; 7 days of oral pre-treatment with taurine did not lessen the bronchoconstriction induced by antigen inhalation, but did subsequently decrease hyperresponsiveness to the bronchoconstrictor 5-hydroxytryptamine, while also decreasing eosinophils and markers of oxidative stress in bronchoalveolar lavage fluid.[174] (Curiously, these effects were quite similar to those which the same group reported with supplemental NAC in the same rodent model – though perhaps not surprising if these agents both promote H_2S synthesis.[86]) More research with taurine in rodent models is evidently warranted.

High-Dose Biotin Can Act as a Direct Activator of Soluble Guanylate Cyclase

An alternative strategy for boosting NO bioactivity in the lung is to administer drugs that interact directly with sGC to stimulate or activate it. A category of drugs known as sGC stimulators binds to the active (reduced) form of sGC, directly enhancing its activity and boosting its responsiveness to NO exposure.[175,176] In contrast, sGC activator drugs interact with the oxidized, deactivated form of sGC (and only that form), restoring its ability to produce cGMP. Drugs of both classes have been shown to reverse the hypersensitivity of airway smooth muscle to bronchoconstrictors in allergic asthma in mice.[177] A sGC stimulator drug, Riociguat, has been approved for treatment of pulmonary hypertension, but has not been studied clinically in asthma.[178]

In concentrations roughly two orders of magnitude higher than the physiological level – readily achievable with affordable supplementation – the vitamin biotin is known to act as an sGC activator, stimulating its production of cGMP 2-3-fold.[179–181] Whether, like pharmaceutical cGMP activators, biotin also potentiates responsiveness of sGC to concurrent NO exposure, has not yet been studied. In spontaneously hypertensive stroke-prone rats, dietary high-dose biotin has been shown to lower blood pressure modestly, while markedly reducing stroke incidence and mortality; concurrent administration of an inhibitor of sGC abrogates the anti-hypertensive effect of biotin in this model.[182] Stimulation of sGC might also underlie the favorable impacts of high-dose biotin on diabetic control demonstrated in rodents and humans, and there is reason to suspect that the clinical utility of high-dose biotin in multiple sclerosis may reflect this mechanism.[183–185] Supplemental biotin is well tolerated in daily doses of 100 mg or more, presumably because the maximal stimulation of sGC it can achieve is far lower than maximal response of this enzyme to

NO.[184,186] Hence, high-dose biotin may have practical potential for use in asthma management – albeit it has never been tested in rodent models of this disorder, or indeed in any lung disorders.

Glycine and Magnesium Promote Bronchodilation via Calcium Modulation

Many tissues express strychnine-inhibitable chloride channels which are opened by interaction with the amino acid glycine. The affinity of these channels for glycine is close to normal plasma concentrations, so elevations of plasma glycine achievable through practical supplementation can increase the open probability of these channels.[187,188] Except in tissues that concentrate chloride intracellularly, glycine-mediated activation of chloride channels exerts a hyperpolarizing effect on plasma membrane by promoting chloride influx. In macrophages – including alveolar macrophages – hyperpolarization reduces their production of oxidants and pro-inflammatory mediators.[187,189,190] However, the effect of glycine on mast cells and eosinophils does not seem to have been studied. Of particular interest is recent evidence that ASM express glycine-activated chloride channels; these induce membrane hyperpolarization in response to glycine.[191] This hyperpolarization opposes calcium influx via voltage-sensitive calcium channels, and hence induces bronchodilation. When patients with cystic fibrosis were given glycine (0.5 g/kg/day in fluid) for 8 weeks in a double-blind cross-over protocol, FEV_1 increased significantly and symptom score improved significantly during supplemental glycine; no adverse effects were noted.[192] Glycine is inexpensive in ample doses, has a pleasant mildly sweet flavor, and is highly and rapidly soluble, lending itself well to administration in water or other fluids; it may also be protective for vascular health.[188] Hence, it may have practical potential as a bronchodilatory nutraceutical in asthma management.

It may also be noted that glycine, like cysteine, is a glutathione precursor, and appears to complement the utility of NAC for raising tissue glutathione levels.[193]

Limited epidemiology suggests that better magnesium (Mg) nutrition correlates with better lung function. In a random sample of over 2600 adults, dietary Mg was assessed by food questionnaire and lung function was assessed; after adjustment for multiple confounding variables, a 100 mg/day increase in Mg intake was associated with a 27 mL higher FEV1, and an 18% lesser chance of showing hyperreactivity in a methacholine challenge.[194] Three placebo controlled studies of Mg supplementation in asthmatics, one involving exclusively children, demonstrated functional and symptomatic improvement during

Table 1 Proposed Nutraceutical Strategies for Controlling Asthma

Nutraceutical	Mode of Action	Dose Range/Day
PhyCB	Inhibit NADPH Oxidase Complexes	100 mg (or 15 g spirulina)
NAC	Support Glutathione Synthesis and Expression of Antioxidant Enzymes:	600 mg, x2-3
Lipoic Acid		600 mg, x2-3
Glycine		5–10 g, x2-3
Selenium		50–100 mcg
Zinc		10–25 mg, x2
Citrulline	Support NO Biosynthesis/Bioactivity:	2–3 g, x2
High-Dose Folate		10–80 mg
High-Dose Biotin		10 mg, x2-3
NAC	Support H2S Biosynthesis:	600 mg, x2-3
Taurine		1–3 g, x2
Glycine	Dilate Bronchioles:	5–10 g, x2-3
Magnesium		100–200 mg, x2

Mg administration;[195–197] one controlled study failed to observe benefit.[198]

The apparent benefit of improved Mg status for lung function in asthma might reflect a direct impact of Mg on ASM hyperreactivity. Agonists which provoke ASM contraction do so, in part, by increasing intracellular free calcium; this activates the calmodulin-dependent myosin light chain kinase (MLCK), which confers a phosphorylation on myosin light chain that promotes contraction. At physiological cellular concentrations, Mg competes with calcium for binding to the N-terminal arm of calmodulin; binding of calcium to two sites on calmodulin is required to induce the conformational change that enables it to activate various enzymes.[199,200] Activation of MLCK by calmodulin is notably less effective when calmodulin is partially Mg-bound.[201] Hence, a small increase in intracellular Mg level may modestly blunt the ability of calcium influx to activate MLCK. Whether this might be the main mechanism whereby Mg status regulates lung function in asthmatics remains unclear.

Overview

This review suggests that nutraceutical measures which help to control lung oxidative stress (PhyCB, NAC, LA or ferulic acid, selenium and zinc), that promote bioactivity of NO (citrulline, high-dose folate, high-dose biotin) and of H_2S (NAC, taurine), and that directly induce bronchodilation via calcium modulation (glycine, Mg) – may have clinical potential for aiding asthma control. Table 1 summarizes these suggestions, with guesstimates as to dose ranges which might be useful. Some of these agents have never been tested even in rodent models of asthma, let alone in clinical asthma, so these proposals are still largely speculative. Nonetheless, if these agents can be shown to be of at least marginal utility, complex supplements or functional foods featuring at least several of them may have practical potential as adjuvants to current management of asthma. Owing to the fact that some of the agents of potential benefit would require bulky multigram daily doses for optimal efficacy (spirulina, citrulline, taurine, glycine), functional foods or beverages might be the most feasible way to deliver them.

Abbreviations

PhyCB, phycocyanobilin; NO, nitric oxide; H2S, hydrogen sulfide; ROCK, Rho-activated kinase; MLCP, myosin light chain phosphatase; C-PKC, protein kinase; C-PLC, phospholipase; sGC, soluble guanylate cyclase; cGMP, cyclic GMP; G-PKG, protein kinase; ASM, airway smooth muscle; BALF, bronchoalveolar fluid; NAC, N-acetylcysteine; LA, lipoic acid; DDAH, dimethylarginine dimethylaminohydrolase; ADMA, asymmetric dimethylarginine; iNOS, inducible NO synthase; MT, Metallothionein; PDE5, phosphodiesterase 5; CBS, cystathionine β-synthase; CSE, cystathionine γ-lyase ; FEV1, forced expiratory volume in one second; Mg, magnesium.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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