

Novel antioxidant approaches to the treatment of upper airway inflammation

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Purpose of review

Current understanding of the role of oxidative stress in airway inflammation suggests that antioxidant therapy may be important to optimize the treatment. This review summarizes recent investigations of novel antioxidant agents for upper airway inflammation, with selected studies focused on lower airway disease as additional candidate therapeutics.

Recent findings

Recently investigated antioxidant therapies for airway inflammation may be broadly grouped into three categories: endogenous metabolic agents, vitamins/nutrients, and botanical extracts. Studies examining effects in upper airway inflammation are limited and primarily consist of in-vitro human and in-vivo animal models. More extensive studies have investigated the benefits of antioxidants in lower airway conditions such as allergic asthma. Existing evidence identifies antioxidant agents with potential therapeutic value, although human studies suggest that subpopulations affected by specific genetic, environmental, dietary factors, or all are most likely to benefit from antioxidant therapy.

Summary

Oxidative stress plays a causative role in upper airway inflammation, and novel strategies to mitigate cellular injury with antioxidant therapy may ameliorate disease in target populations. Preclinical studies demonstrate evidence of anti-inflammatory effects for a number of promising antioxidant agents. Well designed interventional human studies of the upper airway, which account for complex gene–environment–diet interactions, will be necessary to adequately examine the potential clinical benefit of antioxidant therapies for rhinosinusitis.

Keywords

airway inflammation, antioxidants, novel therapeutics, oxidative stress, rhinosinusitis

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Introduction

Substantial evidence implicates oxidative stress as a contributing factor in the pathophysiology of airway inflammation. Few studies [1•,2] illustrate the oxidative effects of diesel exhaust particles (DEPs) and other air pollutants in lower airway inflammation. Consistent with the unified airway model, oxidative stress is also implicated in upper airway inflammation. A growing body of research has examined the effects of antioxidant compounds aimed at preventing or reducing oxidative stress-induced inflammation. Because of the fundamental role of oxidative stress in pulmonary pathophysiology, a preponderance of antioxidant therapeutic research has focused on lower, rather than upper, airway inflammation. The applicability of these findings to the upper airway is currently unknown. This review examines recent studies of antioxidant therapy for upper airway inflammation,

with inclusion of selected antioxidant agents studied in the lower airway as candidate upper airway therapeutics.

Background

Respiratory exposure to air pollutants, pollens, and infectious agents generates oxidative stress with resultant airway inflammation impacting clinical disease [1•,2,3•,4••]. These environmental factors interact with cellular metabolites to create reactive oxygen species (ROS) that oxidize proteins, lipids, and DNA. Excessive reactive species deplete glutathione (GSH) stores and activate intrinsic cellular antioxidant defenses, primarily via the nuclear erythroid 2 p45-related factor 2 (NRF2) signaling cascade. Activation of the antioxidant response element by NRF2 induces expression of phase II metabolizing enzymes that neutralize ROS and include glutathione-S-transferases (GSTM and GSTP), heme

oxygenase 1 (HO1), and NAD(P)H:quinone oxidoreductase (NQO1) [5,6].

When endogenous cytoprotective antioxidant responses are unable to sufficiently reduce the oxidative burden, continued GSH depletion initiates an inflammatory cascade. Signaling via mitogen-activated protein kinase and nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) results in gene transcription of inflammatory cytokines (IL-4, IL-5, IL-13, and TNFα), chemokines, and cellular adhesion molecules [7]. In addition to these direct inflammatory effects, environmentally induced oxidative stress also functions as an adjuvant to enhance Th2 responses to allergen [8].

Current reports [9[•]–13[•]] delineate the role of oxidative stress in the pathophysiology of allergic rhinitis, nasal polyposis, and chronic rhinosinusitis. Recent investigations demonstrate increased leukotriene B4 and 8-isoprostane, markers of oxidative stress, in individuals with allergic rhinitis [11[•]]. Moreover, these effects are augmented by concurrent exposure to environmental tobacco smoke (ETS) [12[•]]. Similarly, new evidence describes the contribution of infectious agents to the

generation of ROS during respiratory infections [4^{••}]. Exhaled and nasal nitric oxide levels are increasingly used as surrogate markers of oxidative stress in studies [14,15^{••},16–21] of upper and lower airway inflammation, although the interpretation and significance of these measurements are complex and beyond the scope of this review.

Antioxidants in the treatment of upper airway inflammation

By definition, antioxidants have the capacity to neutralize ROS. Agents selected for this review show evidence of antioxidant properties as a principal mechanism for the demonstrated anti-inflammatory effects. Several additional novel therapies for airway inflammation possess antioxidant activity but appear to rely on other primary mechanisms for the observed biologic effects [22–24]. Antioxidant and immunomodulatory actions appear intertwined for some novel compounds [25^{••}–27^{••}]. Although established medications for upper airway inflammation may have antioxidant properties, this review will focus on emerging investigations of potential new therapeutics (Table 1) [28–30]. In-vivo human

Table 1 Novel antioxidant therapies for airway inflammation

Antioxidant	Putative mechanism of action	Supportive data for anti-inflammatory effect in airway	Potential therapeutic agents
Enhancement of endogenous metabolic agents			
GSH	Reduction of ROS	Animal (in-vivo) and human (in-vitro/in-vivo) studies	Poor bioavailability of GSH in humans
NAC	Precursor to GSH ROS scavenger	Human (in-vitro) and animal (in-vivo) studies, clinical benefit restricted to select subpopulations	Oral, i.v., inhaled preparations of NAC
HO1	Phase II metabolizing enzyme Potentially decreased TNFα signaling, decreased mucus secretion, or both	Human (in-vitro and genetic association) and animal (in-vivo) studies	Hemin and other HO1 inducers
Vitamins and nutrients			
Vitamins A, C, E, and coenzyme Q10	Antioxidant Other physiologic functions Vitamin C may increase CI secretion in sinus tissue	Human (in-vitro/in-vivo) and animal (in-vivo) studies Clinical benefit for select subpopulations	Foods and oral supplementation
Alpha-lipoic acid	Mitochondrial ROS scavenger	Animal (in-vivo) studies ^a	Foods and oral supplementation
Choline	Endogenous ROS scavenger Precursor to phospholipids and acetylcholine	Animal (in-vivo) studies ^a	Foods and oral supplementation
Botanicals			
Sulforaphane	Phase II enzyme inducer	Human (in-vitro) and animal (in-vivo) studies Human clinical study demonstrating phase II induction in upper airway	Found in cruciferous vegetables
Epigallocatechin 3 gallate	Phase II enzyme inducer Other potential antioxidant effects	Human (in-vitro) study	Found in green tea
Esculetin	ROS scavenger	Animal (in-vivo) studies	Derived from coumarin, found in multiple plant species
PG102	Inducer of HO1 Other potential mechanisms	Human (in-vitro) and animal (in-vivo) studies	Isolated from <i>Actinidia arguta</i> (hardy kiwi)
Caffeic acid phenethyl ester	Inducer of HO1 Inhibits lipoxygenase, cyclooxygenase, NFκB	Animal (in-vivo) studies	Isolated from honeybee propolis

^a In-vivo human studies for other indications. GSH, glutathione; HO1, heme-oxygenase; i.v., intravenous; NAC, *N*-acetylcysteine; NFκB, nuclear factor kappa-light-chain-enhancer of activated B cells; ROS, reactive oxygen species.

studies are lacking for many novel antioxidant agents, likely attributable to issues of bioavailability, unknown safety profiles, and regulatory challenges of human studies to establish clinical efficacy.

Endogenous metabolic agents

Recent studies highlight the potential anti-inflammatory effects of enhancing individual endogenous antioxidant defenses.

Glutathione and N-acetylcysteine

GSH is critical to antioxidant defense of the airway, although the exact role of GSH homeostasis in the pathophysiology of upper airway inflammation remains unclear [31–33]. Recently, Fitzpatrick *et al.* [34•] found decreased GSH levels and increased markers of oxidative stress in the epithelial lining fluid of 65 children with severe asthma as compared with adult controls. Data from animal models continue to illustrate the protective role of GSH in airway inflammation [35•,36•]. Importantly, in contrast to animal studies, oral GSH appears to have low bioavailability in humans [37]. However, other antioxidants, including N-acetylcysteine (NAC) and vitamin C, appear to raise intracellular GSH levels [38–40].

NAC is a GSH precursor and oxygen radical scavenger currently used as oral therapy to minimize hepatotoxicity in acetaminophen overdose. In animal models of allergic sensitization, NAC suppresses the adjuvant effect of DEP and decreases ROS generation by pollen NADPH oxidases, thereby decreasing downstream markers of allergic inflammation [41,42]. Recently, Li *et al.* [26••] demonstrated the adjuvant effects of ultrafine ambient particulates on inflammatory responses using an ovalbumin (OVA)-sensitized murine model. Pretreatment with intraperitoneal NAC abated OVA-specific IgG1 production and inflammatory changes observed in bronchoalveolar lavage fluid (BALF) with ultrafine particulate exposure. Despite the reduction in oxidative stress-associated inflammation, NAC did not prevent the OVA-IgE response.

Small human studies of NAC in upper airway inflammation have been inconclusive (Saxon A., personal communication) and those in lower airway inflammation may demonstrate benefit for selected populations [43–45]. Notably, Moradi *et al.* [46••] recently investigated the effects of NAC treatment therapy on 27 patients with acute lung injury (ALI) and found treatment associated with oxygenation improvement and decreased mortality as compared with untreated controls. Moreover, individuals with GST M1 null, and M1 and T1 double null polymorphisms, that is, the population with potentially increased susceptibility to oxidative stress, had particularly high rates of mortality when untreated.

Heme oxygenase

Increased expression of HO1, a phase II enzyme, has been shown in animal models to abrogate pathological sequelae in various experimental forms of oxidant-induced airway inflammation, including asthma, ozone-induced lung injury, hyperoxia, and influenza infection [47–51]. In conjunction with the ability to neutralize ROS, other protective mechanisms recently attributed to HO1 include attenuated TNF α signaling, inhibition of NF κ B, and decreased expression of adhesion molecules [52••].

HO1 induction with hemin was recently demonstrated to protect against ETS-induced mucus hypersecretion in animal models by interfering with the expression of mucin 5AC (MUC5AC) [53••]. An accompanying human genetic association study found that polymorphisms in the HO1 promoter resulting in low HO1 expression were more common in an ETS-exposed population with chronic sputum production. HO1 levels did not appear to modify outcomes of MUC5AC expression or sputum production in populations without ETS exposure, suggesting that HO1 acts primarily to modify ETS effects.

Two additional known HO1 inducers, curcumin and caffeic acid phenethyl ester (CAPE), have been investigated in preliminary studies [54,55] of airway inflammation. The anti-inflammatory mechanism(s) of action has not been well defined for these agents, and it is unclear whether the antiallergic properties of curcumin are reliant on HO1 induction or other antioxidant effect [56–58]. Recent findings for CAPE are summarized in the section on botanical agents.

Vitamins and nutrients

Vitamins and nutrients with antioxidant properties may have potential therapeutic value in the treatment of airway inflammation.

Choline and alpha-lipoic acid

Choline, a precursor to acetylcholine and an essential component of phospholipids, appears to play a role in modulating oxidative stress [59,60]. α -Lipoic acid is an antioxidant with apparent therapeutic value in neuropathy [61,62]. Both nutrients are available in foods and as oral supplements. Prior studies [63,64] of α -lipoic acid demonstrated promising results with decreased aryl hydrocarbon receptor (AHR) and altered cytokine profiles in animal models of allergic airway inflammation. Recently, Mehta *et al.* [65•] investigated the antioxidant activity of choline and lipoic acid in a murine OVA challenge model. Both intranasal and oral administration of choline, as well as oral administration of lipoic acid,

significantly reduced total lymphocyte counts, total eosinophil counts, eosinophil peroxidase activity, as well as malondialdehyde and 8-isoprostane levels in the BALF.

Vitamins A, C, E, and coenzyme Q10

The effects of vitamin A, C, E, coenzyme Q10, or all supplementation on oxidative stress have predominantly been investigated in lower airway disease.

Human studies

Clinical study outcomes of vitamin therapy for oxidative stress and airway inflammation have generally been disappointing, although select populations may demonstrate benefit as illustrated by a recent study in cystic fibrosis patients [66,67,68–71]. Although serum levels of vitamins A, C, and E reflect augmented intake, it is unclear whether levels in respiratory fluids respond to supplementation [72]. Data from a recent pilot study [73] suggest that supplementation with high-dose vitamin C and α -tocopherol (vitamin E) does not appear to alter levels of vitamin C in sputum. However, Behndig *et al.* [74] demonstrated an increase in concentrations of vitamin C in nasal lavage fluid peaking 2–4 h after ingestion of a high-dose supplement.

In-vitro studies

Although robust clinical studies are lacking, recent in-vitro work on excised sinonasal tissue suggests potentially beneficial effects of vitamin supplementation. Cho *et al.* [75] demonstrated that vitamin C enhances ion transport via the cystic fibrosis transmembrane conductance regulator channel in sinus and nasal epithelial tissue, thereby supporting improved mucociliary clearance. Topical vitamin C administration increased chloride secretion in all tissues, although samples from individuals with chronic rhinosinusitis showed significantly impaired secretory responses to vitamin C as compared with control samples. Thus, impaired ion transport in epithelial cells may contribute to the pathogenesis of chronic rhinosinusitis with high-dose vitamin C potentially improving this deficiency. Other investigators have shown that α -tocopherol treatment decreases DNA damage in oxidative stressed cells from excised nasal epithelial tissue [76]. Similarly, decreased hydrogen peroxide-induced DNA damage was observed in human nasal mucosal cells following administration of coenzyme Q10 and quercetin [77].

Animal models

Murine studies have demonstrated the effects of concurrent administration of vitamins C and E on lower airway allergic inflammation, including decreases in rag-

weed extract-induced ROS levels and associated allergic inflammation [41]. Additional animal studies have investigated effects of various vitamin E preparations (γ and α -tocopherol) on allergic airway inflammation.

An OVA-sensitized rat model demonstrated that 4 days of oral dosing with γ -tocopherol diminished eosinophil infiltration of the tissues of the nose, sinus, and nasolacrimal duct following allergen challenge [78]. Although the upper airway demonstrated dramatic differences with vitamin E treatment, effects on lung tissue eosinophilia were not significant. Similarly, cytokine profiles of the upper and lower airway were incongruous. Nasal expression of IL-4, IL-5, IL-13, and IFN γ by reverse transcriptase (RT)-PCR was significantly inhibited by γ -tocopherol. Pulmonary levels of IFN γ were significantly increased, and other cytokines were unchanged by the treatment regimen.

Mabalirajan *et al.* [79] employed a murine model to measure effects of oral administration of α -tocopherol after sensitization to OVA. Decreases in AHR and Th2 cytokines (IL-4, IL-5, IL-13, eotaxin, and transforming growth factor beta 1), as well as reduction in metabolites of 12 of 15 lipoxygenase, lipid peroxidation, and nitric oxide were noted in animals treated with α -tocopherol. Lung sections also demonstrated a significant reduction in inflammatory cells. Additionally, the authors describe several changes in mitochondria associated with allergen sensitization and exposure that were reversed with vitamin E treatment, including decreased activity of cytochrome *c* oxidase and ultrastructural changes by electron microscopy.

Botanical agents

Antioxidant agents derived from botanical sources have recently been investigated for potential effects on airway inflammation.

Sulforaphane

Sulforaphane and its glucosinolate precursors are found in broccoli sprouts and other cruciferous vegetables. These compounds are naturally occurring potent inducers of phase II metabolizing enzymes shown to have potentially beneficial health effects in animal and human studies [80,81]. An in-vitro study [82] demonstrates that pretreatment with sulforaphane blocks the proallergic effects of DEPs on human B cells. Recently, a placebo-controlled dose-escalation trial [83] was conducted to investigate the in-vivo effects of oral sulforaphane dosing on phase II enzyme induction in the upper airway of humans. GSTM1, GSTP1, NQO1, and HO1 expression was measured in nasal lavage cells by RT-PCR before and after sulforaphane dosing. A statistically significant increase in phase II enzyme expression was noted at

the highest dose of sulforaphane as compared with placebo, illustrating the biologic effects of oral sulforaphane in humans.

Epigallocatechin 3 gallate

Studies [84,85] of epigallocatechin 3 gallate (EGCG), a major component of polyphenols derived from green tea made with *Camellia sinensis*, demonstrate antioxidant capacity in addition to other anti-inflammatory properties. Recently, Kim *et al.* [86[•]] investigated the effects of EGCG on IL-1 β -induced *MUC5AC* gene expression of airway mucin in normal human nasal epithelial cells. Pretreatment of the cells with EGCG-suppressed IL-1 β -induced *MUC5AC* gene expression in a dose-dependent manner, whereas treatment with EGCG alone did not appear to alter baseline expression levels. Additional results suggest that EGCG acts at the *MUC5AC* promoter and inhibits extracellular signal-regulated kinase map kinase phosphorylation.

Esculetin

Esculetin (6,7-dihydroxy-2H-1-benzopyran-2-one) is a naturally occurring coumarin-derived antioxidant isolated from several plant species, including *Fraxinus chinensis* [27^{••},87]. Recently, Mabalirajan *et al.* [79^{••}] demonstrated the antiallergic effects of esculetin administration in an OVA-sensitized murine model with resultant decreases in AHR, lung cotaxin, eosinophils in the BALF, inflammatory cellular infiltrate, and OVA-specific IgE. Moreover, esculetin reduced the expression of 8-isoprostane and other metabolites of 15 lipoxygenase and lipid peroxidation. Similar to findings with α -tocopherol, the authors demonstrate that esculetin treatment reversed allergen-induced detrimental changes to mitochondria [79^{••}].

PG102

The antiallergic properties of PG102, a water-soluble extract isolated from *Actinidia arguta* (hardy kiwifruit), have recently been investigated by Kim *et al.* [25^{••}] using human B and T-cell lines and a murine OVA sensitization model. *In vitro*, PG102 reduced the levels of IgE, IL-5, and IL-13. PG102 administration in the murine experiments decreased eosinophils and IL-5 in BALF and reduced AHR. Early evidence supports HO1 induction as the mechanism of action for PG102 effects.

Caffeic acid phenethyl ester

CAPE, a component of honeybee propolis, is an antioxidant with putative benefit in a variety of disorders. CAPE induces HO1, but also appears to inhibit lipoxygenase, cyclooxygenase, and NF κ B [55,88–90].

Recently, the administration of CAPE in an OVA-sensitized murine model of allergic asthma reduced ROS in the BALF and decreased effects of allergic sensitization, including reductions in AHR, inflammatory cells in the BALF, pathological changes in the lung tissue, and OVA-specific IgE [58].

Genetic susceptibility

Variability in oxidative stress susceptibility is an essential consideration for future human investigations of antioxidant therapy for airway inflammation. Polymorphisms of inducible phase II antioxidant enzymes (NQO1, GSTM1, and HO1) and related genes governing the host response to oxidative stress likely confer differing capacities to mitigate ROS in both the upper and lower airway. This genetic susceptibility to oxidative stress is demonstrated in host variability to the proallergic effects of oxidative air pollutants [2,91,92[•]]. In particular, polymorphisms in NQO1, GSTM1, and GSTP1 have been associated with modified risk and clinical manifestations of asthma in human studies on the effects of ozone [69,93[•],94]. Similarly, polymorphisms in GSTM1 and GSTP1 have a significant effect on nasal inflammatory responses with concomitant ragweed pollen and DEP exposure in ragweed-allergic individuals [95].

Genetic heterogeneity in response to oxidative stress may partially explain the generally equivocal results of human clinical studies examining antioxidant therapy for airway inflammation. Rare studies designed to examine genetically susceptible subpopulations exposed to excessive oxidative stress have suggested clinical benefit of antioxidant therapy for airway inflammation. Polymorphisms in GSTM1 have been associated with benefit from antioxidant therapy in studies of pediatric asthma patients in Mexico City [69]. Data from Moradi *et al.* [46^{••}] discussed above suggest that individuals with GSTM1 null and GSTM1, GSTT1 null genotypes may particularly benefit from NAC administration for ALI/acute respiratory distress syndrome (ARDS). Genetic polymorphisms may also interact with dietary factors to determine oxidative stress susceptibility, response to antioxidant therapy, or both. Pediatric asthma patients with low intake of dietary vitamin E appear to benefit from vitamin E supplements with protective effects against the acute nasal response to ozone exposure [71]. A recent study [96[•]] from Toronto found individuals with GSTM1 and GSTT1 null genotypes are at increased risk of vitamin C deficiency if dietary intake is suboptimal, suggesting a 'gene–diet' interaction.

Conclusion

The rationale for antioxidant therapy is supported by considerable evidence for oxidative stress in the

pathophysiology of airway inflammation. However, limited studies have examined the anti-inflammatory effects of these agents in the upper airway. To date, investigations have predominantly focused on the lower airway to identify beneficial strategies for treating pulmonary disease. These results may provide candidate therapies for the upper airway, although some evidence suggests differential responses of antioxidant therapy in the lower vs. upper airway. In addition, the relevance of in-vitro and animal efficacy data to human upper airway inflammatory conditions is unknown.

Well designed interventional human studies of the upper airway will be necessary to establish the clinical benefit of antioxidant therapies for rhinosinusitis. Complex interactions between genetic polymorphisms, environmental exposures, and dietary intake add to the difficult task of investigating the potential clinical benefits of these novel agents. However, given the high prevalence of rhinosinusitis, its detrimental impact on quality of life, and continued population exposures to prooxidant air pollutants, focused efforts to develop promising antioxidants as upper airway therapeutics may greatly improve public health.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000–000).

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