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Immune-Enhancing Role of Vitamin C and Zinc and Effect on Clinical Conditions

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Vitamin C · Zinc · Oxidative stress · Effects on immune response · Risk of infections

Abstract

Vitamin C concentrations in the plasma and leukocytes rapidly decline during infections and stress. Supplementation of vitamin C was found to improve components of the human immune system such as antimicrobial and natural killer cell activities, lymphocyte proliferation, chemotaxis, and delayed-type hypersensitivity. Vitamin C contributes to maintaining the redox integrity of cells and thereby protects them against reactive oxygen species generated during the respiratory burst and in the inflammatory response. Likewise, zinc undernutrition or deficiency was shown to impair cellular mediators of innate immunity such as phagocytosis, natural killer cell activity, and the generation of oxidative burst. Therefore, both nutrients play important roles in immune function and the modulation of host resistance to infectious agents, reducing the risk, severity, and duration of infectious diseases. This is of special importance in populations in which insufficient intake of these nutrients is prevalent. In the developing world, this is the case in low- and middle-income countries, but also in subpopulations in industrialized countries, e.g. in the elderly. A large number of randomized controlled intervention trials with intakes of up to 1 g of vitamin C and up to 30 mg of zinc are available. These trials document that adequate intakes of vitamin C and zinc ameliorate symptoms and shorten the duration of respiratory tract infections including the common cold. Furthermore, vitamin C and zinc reduce the incidence and improve the outcome of pneumonia, malaria, and diarrhea infections, especially in children in developing countries.

Nutritional and Physiological Aspects of Vitamin C and Zinc

Vitamin C (ascorbic acid) and zinc are essential nutrients and play important roles in nutrition and maintenance of human health. Both have profound effects on cellular growth and differentiation, and are vital for the optimal functioning of the immune system. Inadequacy and clinical deficiency of vitamin C and/or zinc lead to impaired immune response with altered resistance to infections, impaired growth, and weakened collagenous structures with delayed wound healing.

The nutritional and physiological aspects of these essential nutrients have been widely reviewed and a large number of comprehensive publications on vitamin C [1, 2] and zinc [3, 4] are available. Therefore, the nutritional and physiological aspects of these essential nutrients are only briefly summarized here.

Vitamin C has direct antioxidant capacity and contributes to the protection of cells from the damaging effects of endogenously produced or exogenous reactive

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oxygen radicals and reactive nitrogen species, e.g. during immune activation. Vitamin C was shown to protect neutrophils from reactive oxygen species generated during phagocytosis [2], to prevent endogenous oxidative damage to lymphocytes and sperm DNA [5–7], and vitamin C supplementation decreased significantly H_2O_2-induced DNA damage in lymphocytes in healthy male and female nonsmokers [5]. Vitamin C prevents oxidative damage to lipids, proteins, and DNA, which has been implicated as a major contributing factor in the development of chronic diseases such as cardiovascular disease, cancer, and cataract, respectively [2, 8]. Evidence further suggests that vitamin C provides indirect antioxidant protection by regenerating other biologically important antioxidants such as glutathione and vitamin E to their active state [9]. Vitamin C functions as an electron donor involving either monooxygenase or dioxygenase activities in a number of important enzymes: dopamine-β-hydroxylase (biosynthesis of the catecholamines norepinephrine and epinephrine), peptidyl-glycine monooxygenase (amidation of peptide hormones) and is involved in tyrosine metabolism (4-hydroxyphenylpyruvate dioxygensase) [1, 2]. Vitamin C further acts as a cofactor for hydroxylases and oxygenase metalloenzymes. Proline and lysine hydroxylase catalyze the posttranslational hydroxylation of peptide-bound proline and lysine residues, which is essential for the development of functionally active collagen in its triple helical structure and thus for effective wound healing [10]. Further, it functions as a reducing agent for mixed-function oxidases, which are of importance in the drug-metabolizing system in the microsomes, and as a consequence, in vitamin C deficiency, the activity of the drug-metabolizing enzymes and the cytochrome P-450 electron transport are lowered [2]. Ascorbic acid stimulates non-hem iron absorption from the intestine and modulates iron transport and storage [11].

Zinc is important in cellular growth and differentiation with profound effects on antioxidant defense, collagen synthesis, and the immune system [3, 4]. A large number of enzymes depend on zinc for catalytic activity (e.g. alcohol dehydrogenase; zinc containing metallo-enzymes such as RNA polymerases, carbonic anhydrase, and alkaline phosphatases), and removal of zinc results in loss of the enzymatic activity without affecting the enzyme protein irreversibly [12]. By its antioxidant capacity, zinc contributes to the protection of cells from the damaging effects of reactive oxygen radicals and reactive nitrogen species produced during e.g. immune activation. Zinc antagonizes the catalytic properties of the redox-active metals iron and copper with regard to the formation of hydroxyl radicals from H_2O_2 and superoxide by competing with certain types of binding sites for copper and iron, and blocking the initiation of destructive processes [13].

Elevated levels of superoxide anions cause increased formation of reactive oxygen species that can damage lipids, proteins and DNA which has been implicated as a major contributing factor in chronic diseases such as cardiovascular disease, cancer and cataract [8]. The antioxidant properties of zinc have recently been reviewed [13]. The antioxidant enzyme Cu, Zn-superoxide dismutase catalyzes the reduction of two superoxide anions to molecular oxygen and hydrogen peroxide. However, in this enzyme, copper provides the catalytic activity whereas the role of zinc is structural. Zinc is essential for the intracellular binding of tyrosine kinase to T cell receptors, which are required for T lymphocyte development and activation [14, 15]. The mechanism that underlies the antioxidant action causes acute and chronic effects. The acute effect may involve protection of protein sulfhydryl groups against oxidation and oxidative damage to membranes, or reduction in the formation of hydroxyl radicals from H_2O_2 through the antagonism of redox-active transition metals such as copper and iron [16]. Thiol-dependent enzymes and proteins containing thiol groups have been shown to be protected by zinc from oxidation, e.g. δ-aminolevulinate dehydratase, DNA-zinc binding proteins (zinc fingers) or tubulin [13]. Studies in zinc-deficient animals and cell systems have shown an increased free radical production and enhanced injury from exposure to oxidative stress [14].

Zinc is important for structural integrity by facilitating protein folding to generate biologically active molecules. Structural sites or zinc fingers have a broad cellular distribution and are present in transcription and replication factors, in nuclear hormonal receptors, and in signal transduction factors. Examples for zinc finger transcription factors are retinoic acid receptors and vitamin D receptors. Nuclear hormone receptors include those for estrogen, testosterone, and vitamin D. Zinc finger proteins have a broad cellular distribution and are involved in protein-protein interaction affecting cellular differentiation and proliferation [17]. Removal of zinc from zinc finger proteins with apometallothionein in vitro has been shown for a transcription factor to result in loss of function [18]. Zinc fingers contribute to zinc requirement and support the fundamental and critical physiological role of zinc, such as the intracellular binding of tyrosine kinase to T cell receptors, which are required for T lymphocyte development and activation [19, 20].

This overview attempts to demonstrate that sufficient intakes of vitamin C and zinc are essential to support adequate immune response, to elaborate on the clinical consequences, such as the risk, severity and duration of infectious diseases, and to demonstrate the relevance of the immune response modulated by these nutrients to various population groups.

Immune Function and Oxidative Stress

The immune system protects the organism from invasion and damage from a wide range of microorganisms, such as viruses, bacteria, fungi or parasites, by a highly complex biological response, which involves cellular proliferation, enhanced protein synthesis and inflammatory mediator production as well as physiological changes. In the activation of the immune system, the process of cell proliferation is most active among T lymphocytes. Concurrently, protein synthesis, immunoglobulin synthesis by B lymphocytes, and acute phase protein synthesis in the liver are enhanced. Inflammatory mediators such as the proinflammatory cytokines (IL 1; IL 6) and tumor necrosis factor-α (TNF-α), prostaglandins, leukotrienes, and reactive oxygen and nitrogen species, are increased [21].

The generation of reactive oxygen species is part of the physiological function of cells involved in host defense, such as activated neutrophils and macrophages, especially during chemotactic locomotion and phagocytosis. The reactive oxygen species play an essential role in the intracellular killing of bacteria and other invading organisms. Whereas this microbicidal activity is a beneficial activity, on the other side the immune system is particularly vulnerable to oxidative stress, since immune cells rely on cell-cell communication via membrane receptors and any damage to the signaling systems has been shown to impair the ability to build up an immune response [22, 23]. However, besides the antimicrobial and therefore protective properties of these oxidants, other biomolecules may be vulnerable to free radical attack. Especially, cell membranes rich in long-chain polyunsaturated fatty acids are susceptible to oxidative destruction, which could lead to a loss of membrane integrity, altered membrane fluidity, and may result in alterations of cell-cell communication, herewith contributing to degenerative disorders such as cancer and cardiovascular diseases [8]. Antioxidants, such as vitamin C and zinc, could play an immunomodulatory role by preventing tissue damage mediated by immune-system-generated reactive oxidant species, especially in population groups with poor dietary habits. Marginal or clinical nutrient deficiency may impair the proper functioning of the immune system, suppressing various immune functions, which are critical determinants of host resistance. As a consequence, the deficient individual becomes more susceptible to infections. In many cases, by supplying the deficient nutrient, immune function may be normalized and resistance to infections restored [22, 23].

Furthermore, vitamin C is involved as an antioxidant in the maintenance of the endothelial function, mainly by preventing or reversing endothelial dysfunctions leading to the development of cardiovascular diseases [24–26]. Mechanisms discussed include suppression of the induction of endothelial cell apoptosis [24], protection of nitric oxide against oxidative inactivation [26], stimulation of nitric oxide synthesis [27], intracellular reduction of oxidized glutathione [28], and prevention of the formation of atherogenic oxidized phospholipids [29].

Vitamin C and the Immune System

The high cellular concentration of vitamin C and its rapid decline in plasma and leukocytes during stress and infection suggest a role in the process of immune response [30–32]. Vitamin C was found to be a stimulant of leukocyte functions, especially of neutrophil and monocyte movement. Supplementation of healthy adults (1–3 g/day) and children (20 mg/kg/day) enhanced neutrophil chemotaxis in vivo, but bactericidal activity was not enhanced [32]. Lymphocyte proliferation was not impaired in vitamin C deficiency in humans and the number of circulating CD4+ or CD8+ cells was not altered [33]. Vitamin C in doses of 1–5 g/day for 3 days and over several weeks increased human T lymphocyte proliferation and neutrophil motility towards lipopolysaccharide-activated autologous serum [34, 35]. In vitro treatment of peritoneal macrophages with antioxidant vitamins, including vitamin C, was shown to stimulate the entire process of phagocytosis. The observed decrease of vitamin C in plasma and leukocytes during infective periods suggests that the increased generation of oxidizing agents is counteracted by reaction with vitamin C, and herewith the host is protected against any harmful oxidative action [31].

In studies in healthy subjects, administration of vitamin C resulted in improvement of several components of human immune parameters, such as antimicrobial and natural killer cell activities, lymphocyte proliferation,

chemotaxis, and DTH [35–38]. The effect of vitamin C intake with the diet on immune function was studied in young, healthy nonsmokers. The volunteers consumed a vitamin-C-deficient diet and then increased their vitamin C intake from 5 to 250 mg/day. Upon ingestion of the vitamin-C-deficient diet, plasma and leukocyte vitamin C concentrations were decreased by about 50%, and DTH response to several antigens was decreased as well. With higher doses (60 and 250 mg/day) the DTH response was normalized, but lymphocyte proliferation was not affected [37]. In older people, known to have reduced vitamin C plasma and leukocyte concentrations even if they are not institutionalized, vitamin C supplementation resulted in enhanced cell-mediated immunity [39]. Intracellular concentrations of vitamin C in human leukocytes have been shown to decline with increasing age, accompanied by neutrophil function impairment. Oral administration of vitamin C resulted in improved neutrophil functions and serum immunoglobulin levels [40]. An earlier investigation showed that administration of 1 g of vitamin C together with 200 mg of vitamin E for 16 weeks to healthy elderly women enhanced neutrophil chemotaxis and phagocytosis, and decreased concentrations in serum lipid peroxides, which is indicative of improved resistance to oxidative stress [41]. Other studies, however, did not show any alterations in indices of immune function following vitamin C administration, which may be due to the fact that individuals participating in these studies had already adequate vitamin C baseline concentrations [1].

Vitamin C stimulated interferon production in vitro when incubated with cultured mouse cells and in vivo when administered to mice [42]. There is evidence that ascorbic acid may also have antiviral activity in vivo [43, 44]. Topical application of ascorbic acid in patients with herpes simplex virus infections decreased the duration of the lesions and viral shedding [45].

Zinc and the Immune System

The immune-related functions of zinc have been reviewed recently [46]. The innate or nonspecific immunity as the first line of defense is disturbed by altered zinc concentrations. Lowered zinc status, such as in subclinical deficiency and zinc deficiency impairs cellular mediators of innate immunity such as phagocytosis by macrophages and neutrophils, natural killer cell activity, generation of the oxidative burst, and complement activity [47]. These alterations are considered to be important contributors to increased susceptibility to infections, especially during childhood. Patients on total parenteral nutrition without zinc supplementation showed reduced resistance to infections that was corrected by addition of zinc to their nourishment [48]. Zinc plays an essential role in cell-mediated and humoral immunity, as observed in in vitro studies and in studies in zinc-deficient subjects [46, 49].

Consistent findings in zinc deficiency are a decrease in lymphocyte numbers (lymphopenia), impaired lymphocyte development, reduced proliferation, increased apoptosis, and thymic atrophy [50]. Zinc deficiency in experimental animals is associated with low thymic weight, a progressive loss of T lymphocytes and macrophages, delayed hypersensitivity and cytotoxic activity, impaired B and T cell function, and reduced antibody recall responses. Zinc is an essential cofactor for the thymic hormone thymulin, which induces several T cell markers, and promotes T cell function, including allogenic cytotoxicity, suppressor functions, and interleukin-2 production. It also modulates cytokine release by peripheral blood nuclear cells and induces the proliferation of CD8+ T cells, which function as cytotoxic cells able to recognize and kill pathogens [49]. In experimentally induced zinc deficiency, subjects show low serum thymulin activity, impaired T cell and natural killer cell activities, and decreased interleukin-2 and interferon production [51].

Vitamin C and Zinc and Infectious Diseases

Vitamin C and zinc have been demonstrated to support important functions, which are essential in maintaining health. Especially, their role in modulating host resistance to infectious agents is considered of importance. Thus, undernutrition and deficiency of these nutrients may already result in profound effects on overall immune function with increased susceptibility to oxidative stress and subsequent respiratory infections. Micronutrient deficiencies are quite common in the elderly and even apparently healthy elderly, e.g. in European countries, were shown to have low dietary intake of at least one nutrient [52]. Vitamin C intakes were reported in the SENECA study to be insufficient in about 5–10% of the elderly population in Europe [53]. But, also heavy chronic smokers, exposed to continuous increased oxidative stress, are known to have a reduced vitamin C status. The NHANES III study showed significantly lower leukocyte vitamin C concentrations in smokers than in nonsmokers [54]. This may be of importance, since the immune system also becomes activated by environmental pollutants,

burns, injury, exposure to radiation, and the presence of chronic inflammation. The proinflammatory cytokines will indirectly ensure that vitamin C, and other antioxidants, are released from host tissues and made available for antioxidant defense. If not re-supplied, the host is at risk of developing a deficiency state.

Similarly to vitamin C, the body has no storage system for zinc; a steady intake of zinc is thus necessary. Zinc deficiency in developed countries is rather seldom, but risk groups for zinc undernutrition and zinc deficiency include vegetarians (decreased absorption), elderly (insufficient dietary intake), patients with intestinal diseases (causing decreased absorption), children, pregnant and nursing women, and patients with chronic infections or inflammatory diseases often seen especially in the elderly (increased requirement) [55]. The NHANES III study in the US reported groups at greatest risk of inadequate zinc intake to be children aged 1–3 years, adolescent females aged 12–19 years, and elderly people aged >71 years [56]. In developing countries, infections very often coexist with multiple nutritional deficiencies which may be the result of general malnutrition. Especially children, in the case of zinc, and the elderly for both nutrients are affected.

Vitamin C as well as zinc supplementation has been shown to be of therapeutic value in specific clinical conditions. In Chediak-Higashi syndrome, which is characterized by clinical phagocytic cell dysfunction, in chronic granulomatous disease, and in recurrent furunculosis, vitamin C supplementation increases neutrophil chemotaxis, improves bactericidal activity and reduces the length of clinical illness [36, 57, 58]. The rare autosomal recessively inherited disease acrodermatitis enteropathica, caused by impaired absorption of zinc from the gastrointestinal tract, is characterized by dermatitis, intermittent diarrhea, recurrent infections, growth retardation, impaired immune function and increased susceptibility to infections [59]. Zinc deficiency was shown to result in impaired immune function and increased susceptibility to infections [60].

Respiratory Infections and the Common Cold

Based on its immunostimulatory properties, vitamin C has been postulated to be effective in ameliorating symptoms of upper respiratory tract infections, especially the common cold, and a large number of placebo-controlled studies in large cohorts have been carried out to evaluate the potential role of vitamin C supplementation in their prevention of the common cold. The most recent overall review evaluated whether oral vitamin C in doses of 200 mg or more daily reduces the incidence, duration or severity of the common cold when used either as continuous prophylaxis or as therapy after the onset of symptoms [61]. A meta-analysis of 29 trials involving 11,077 study participants indicated that routine prophylaxis with high-dose vitamin C in the normal population did not result in a reduction in the incidence of colds (RR 0.96; 95% CI 0.92–1.00), whereas a subgroup of 6 trials involving 642 marathon runners, skiers, and soldiers on sub-arctic exercise showed a 50% reduction in the risk of colds (RR 0.50; 95% CI 0.38–0.66). A consistent and statistically significant benefit in reduction of cold duration during prophylaxis with vitamin C of 8% (95% CI 3–13%) for adults and 13.5% (95% CI 5–21%) for children was observed in 30 comparisons involving 9,676 respiratory episodes. The meta-analysis of severity of episodes while on prophylaxis with vitamin C was based on 15 trial comparisons with 7,054 respiratory episodes. When taking days confined to home and off work or school as measure of severity ($p = 0.02$) or including only studies which used symptom severity scores ($p = 0.16$), the pooled results revealed a difference favoring vitamin C prophylaxis, which is of great economic importance. No significant difference in cold duration or cold severity was seen versus placebo during therapy with up to 4 g of vitamin C when started at onset of cold symptoms, but one large trial reported benefit from an 8 g therapeutic dose at onset of cold symptoms. The authors concluded that prophylactic vitamin C supplementation may not be justified for community use, but could be of benefit in persons exposed to brief periods of severe physical exercise and/or cold environment. The consistent and statistically significant small benefits for duration and severity in those on regular vitamin C prophylaxis point to a role for vitamin C in respiratory defense mechanisms [61]. Elderly patients hospitalized with acute respiratory infections (pneumonia; chronic bronchitis) showed a better overall status measured as a 'total respiratory clinical score' when receiving 200 mg/day of vitamin C over 4 weeks [62].

Following exercise, increased reactive oxygen species are produced that may exceed antioxidant defense, resulting in oxidative damage and the stimulation of an inflammatory response with increased proinflammatory cytokine production. Ultramarathon runners may therefore benefit from antioxidant supplementation. Subjects supplemented with 600 mg vitamin C per day showed a significant decrease in the incidence of postrace upper respiratory infections and were able to cope better with the oxidative stress response resulting from strenuous exercise [63]. In a more recent study, it was shown that anti-

oxidant supplementation of a mixture of 1 g vitamin C and 300 mg vitamin E over 6 weeks prevented exercise-induced oxidative stress, measured as F2-isoprostane as marker for lipid peroxidation [64].

Earlier in vitro studies have shown that zinc salts, at concentrations of about 0.1 mmol/l, possess antiviral properties and inhibit rhinovirus replication [65]. The effects of zinc on rhinovirus replication were found to be related to the concentration of Zn^{2+} ions and were unrelated to total amount of zinc. The antiviral effect of Zn^{2+} ions was found to be as effective as that of interferon [66]. Alternatively, zinc salts may protect plasma membranes against lysis by cytotoxic agents, such as microbial toxins [67].

On the basis of antiviral interaction, several randomized and placebo-controlled clinical trials were carried out, mainly with lozenges of zinc gluconate. Several meta-analyses were conducted, including 8 clinical trials [68], and 7 clinical trials [69]. The summary odds ratio for the presence of any cold symptoms at day 7 was 0.50 (95% CI 0.19–1.29), and the authors concluded that convincing evidence for the effectiveness of zinc gluconate lozenges in reducing the common cold is lacking, but methodological flaws and small sample size made a definitive conclusion difficult [68]. The meta-analysis by the Cochrane Collaboration also concluded that there was no convincing evidence for zinc gluconate lozenges, since 4 trials have shown that zinc may be effective in the treatment of the common cold by reducing the duration and severity of symptoms, whereas 4 other trials have shown no benefit. But it was stated that due to difficulties with respect to blinding and bioavailability of zinc from the lozenges better designed studies are necessary before a final conclusion can be drawn [69]. Whereas most trials were carried out on patients with community-acquired infections, in two studies volunteers were inoculated with rhinovirus, but the authors reported opposite findings [70, 71]. As a treatment, zinc gluconate lozenges (containing 23 mg; 1 lozenge/2 waking hours for a total of 12 lozenges/day for 4.5 days; 12 volunteers) shortened experimentally induced rhinovirus-2 colds by a statistically significant average of 4.8 days [70]. The other trial was carried out in 55 individuals with zinc gluconate lozenges (containing 23 mg of zinc gluconate and 90 mg citric acid; 1 lozenge every 2 h for a total of 8 lozenges/day for 5 days). The treatment actually was found to prolong the cold by 1 day compared to placebo [71].

Since the Cochrane review, three trials have been reported [72–74]. Prevention and treatment of rhinovirus infection with intranasal zinc gluconate in 91 subjects did not show any effect [72]. The efficacy of zinc acetate and zinc gluconate lozenges in a study on 273 subjects challenged with rhinovirus and in 281 patients with spontaneous colds treated by intranasal application of zinc gluconate significantly reduced the duration of the cold from 3.5 to 2.5 days in the rhinovirus-challenged group versus placebo. No effects were seen in the zinc acetate group. Likewise, no effect was observed on duration and severity with both treatments in those subjects with spontaneous colds [73]. In the third study, the effect of zinc acetate in reducing the duration of symptoms of the common cold was investigated in a randomized, double-blind, and placebo-controlled trial in 50 volunteers. The participants were recruited within 24 h of developing symptoms and received zinc acetate lozenges containing 12.8 mg of zinc acetate every 2–3 h while awake as long as the symptoms prevailed. Measurements recorded daily for 12 days included subjective scores for sore throat, nasal discharge, nasal congestion, hoarseness, muscle ache, fever, and headache. Compared with the placebo group, the zinc acetate group showed a significantly shorter overall duration of cold symptoms (4.5 vs. 8.1 days; $p < 0.005$), cough (3.1 vs. 6.3 days), nasal discharge (4.1 vs. 5.8 days) and significantly decreased total severity scores for all symptoms ($p < 0.002$). The authors concluded that zinc acetate may be efficacious in reducing the duration of common cold symptoms and should be preferred over zinc gluconates [74].

Overall, the available trials on the effect of zinc on common colds report conflicting results. However, the observation that prospective efficacy of zinc lozenges might be predicted based on the zinc ion availability from chemically different zinc lozenge formulations, may at least partly explain the differences in clinical outcome [75]. Zinc ion availability identifies the potential for absorption of Zn^{2+} ions at physiological pH 7.4 into oral and oropharyngeal mucosal membranes, which is influenced by components in the lozenges (chelating substances; food acids). The analyses of zinc lozenge formulations used with regard to their zinc ion availability showed a linear relationship with the reduction in the duration of common colds in days, and suggests that the lozenges containing zinc acetate without substances minimizing zinc ion availability (e.g. chelators such as citric acid or tartaric acid, EDTA, amino acids; salicylic acid) should be investigated, since 100% of zinc acetate is released as Zn^{2+} ions at physiological pH [75]. Unfortunately, these aspects were not considered so far by any meta-analysis of the effect of zinc on the common cold. However, the recent effective trial with zinc acetate lozenges to reduce

duration and severity of symptoms actually seems to support the importance of the availability of zinc ions [74]. Thus, further carefully conducted clinical trials are necessary to prove or disprove the efficacy of zinc acetate lozenges against the common cold, and such trials may be of high economic value. Nevertheless, the mechanism underlying a potential effect of zinc acetate in the treatment of the common cold is still unclear.

The Zinc Investigators' Collaborative Group [76] evaluated the effect of zinc supplementation in doses ranging from 10 to 30 mg/day in children aged 3–36 months, on the incidence of pneumonia in a meta-analysis including 4 randomized controlled trials and found a 41% reduction (95% CI 17–59%). This finding was confirmed in children, in whom recent trials showed a 26% reduction in the risk of pneumonia [77]. The revised pooled analysis of all 5 trials showed a 34% reduction in the incidence of pneumonia infections (95% CI 17–47%) [76, 78]. Zinc supplementation together with antibiotics resulted in a 30% reduction versus placebo with regard to the duration of severe pneumonia and individual markers of disease severity (fast breathing, hypoxia), and a mean reduction of 25% with regard to the duration of hospital stays [79]. A benefit of daily zinc supplementation to Bangladeshi children with diarrhea (20 mg for 14 days) showed a statistically nonsignificant downward trend in the incidence and in hospital admission for acute lower respiratory infection in the intervention group [80]. A recent study showed that zinc treatment (10 mg zinc as acetate twice daily for 4 days) of 153 children aged 2–24 months with acute respiratory infection significantly reduced the duration of fever ($p = 0.003$) and very ill status ($p = 0.004$) in boys, but not in girls [81].

Effect of Nutritional Status

Viral and bacterial infections are the most common causes of acute diarrhea. Worldwide, acute infectious diarrhea has a huge impact, causing over 5 million deaths per year. The clinical condition of diarrhea does not seem to be related to decreased vitamin C status. Zinc deficiency is highly prevalent in children in developing countries due to inadequate dietary intake, lack of intake of animal foods, and reduced bioavailability of zinc due to a high phytate:zinc ratio in the diet [82]. The adverse effects of zinc deficiency on the immune response are likely to increase the susceptibility of children to infectious diarrhea, and chronic or persistent diarrhea may further compromise the zinc status and many children become zinc deficient due to increased fecal losses of zinc during diarrhea [83].

Diarrhea is clearly established to increase the rate of loss of endogenous zinc from the intestinal mucosa [84]. Results are available from a large number of randomized controlled intervention trials in developing countries assessing the effect of zinc supplementation in the prevention of diarrhea. The Zinc Investigators' Collaborative Group performed a pooled analysis of trials in children to assess the effects of zinc supplementation in the prevention of diarrhea and pneumonia and found an 18% reduction in the incidence of diarrhea. Zinc also had a positive therapeutic effect in the treatment of acute and persistent diarrhea (34 and 27% reduction, respectively). Overall, zinc was found to have significant therapeutic effects in persistent diarrhea by decreasing the duration of episodes, lowering stool frequencies, and reducing treatment failures or deaths by 40%. These trials were conducted in children aged between 6 months and 3 years and zinc treatment ranged from 10 to 30 mg/day [76, 78]. Subsequent trials in Bangladesh [85, 86], India [87, 88], and Brazil [89] replicated these findings. It is thus suggested that zinc supplementation could be an important adjuvant therapy for treating acute diarrhea in children in developing countries [85].

Zinc is essential for several lymphocyte functions which have been related to resistance to malaria infections, such as production of immunoglobulin IgG, interferon-γ, and tumor necrosis factor-α, and just like vitamin C, it is also most important in the microbicidal activity of macrophages [31, 49]. In children with acute malaria infection, baseline plasma zinc concentrations are very low (<9.2 μmol/l) and were found to be inversely correlated with C-reactive protein and the degree of parasitemia [90]. Cross-sectional studies showed an association between low zinc status and increased incidence of malaria [91]. The Zinc Against Plasmodium Study Group evaluated the therapeutic effect of zinc administered as an adjuvant to standard treatment in large double-blind controlled trials and was not able to find any effect [92], whereas zinc supplementation in patients suffering from malaria infections showed on average a 36% decrease in the incidence of febrile illness [93].

Conclusion

Adequate intakes of vitamin C and zinc are essential for health. These nutrients interact with the human immune system by supporting immune responses and by providing antioxidant protection to exogenously derived and endogenously generated reactive oxygen species pro-

duced during the inflammatory response. Vitamin C stimulates neutrophil chemotaxis and contributes to maintaining the redox integrity of cells thereby protecting them against reactive oxygen species. The latter are generated during the respiratory burst to kill pathogens and are elevated in the inflammatory response. Likewise, zinc undernutrition or deficiency was shown to impair cellular mediators of innate immunity such as phagocytosis, natural killer cell activity, and the generation of oxidative burst.

Both vitamin C and zinc have been investigated to determine their role in the amelioration of the common cold. Zinc salts as lozenges have been investigated for their potential therapeutic effect on the common cold on the basis of their direct anti-viral activity. Available trials on the effect of oral administration of zinc salts as lozenges reported conflicting results and the available evidence is inconclusive. However, a recent therapeutic trial with zinc acetate showed a significant reduction in the overall duration of symptoms and overall severity score. The discrepancies in clinical outcome with zinc salts on the common cold have recently been suggested to be due to the different zinc ion availability from these formulations to the oral and oropharyngeal mucosal membranes. Therefore, more studies are required, especially with zinc acetate.

The current belief is that regular prophylactic intakes of vitamin C at doses of 200 mg or more daily have no effect on the incidence of the common cold, but may be beneficial in the reduction of the severity and duration of the symptoms, suggesting that vitamin C plays some role in the respiratory defense mechanisms. However, the elderly, who have been shown to have a lowered vitamin C status and may therefore be more prone to infections, persons exposed to continuous oxidative stress, such as chronic smokers, and persons exposed to heavy physical exercise and/or cold environment may benefit from a moderate continuous vitamin C intake.

Other vulnerable population groups include children. Due to the high prevalence of zinc deficiency, especially in children in developing countries, and to the impaired immune status, susceptibility to infectious diarrhea, malaria, and pneumonia is found to be substantially increased. Large intervention trials with daily intakes of 10–30 mg of zinc have shown that zinc supplementation could be an important adjuvant therapy for treating these infectious diseases in children in developing countries.

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