Duration and Severity of Symptoms and Levels of Plasma Interleukin-1 Receptor Antagonist, Soluble Tumor Necrosis Factor Receptor, and Adhesion Molecules in Patients with Common Cold Treated with Zinc Acetate

Ananda S. Prasad,[1] Frances W. J. Beck,[1] Bin Bao,[1] Diane Snell,[1] and James T. Fitzgerald[2]

[1]Wayne State University School of Medicine, Detroit, and [2]University of Michigan Medical School, Ann Arbor

Background. Zinc lozenges have been used for treatment of the common cold; however, the results remain controversial.

Methods. Fifty ambulatory volunteers were recruited within 24 h of developing symptoms of the common cold for a randomized, double-blind, placebo-controlled trial of zinc. Participants took 1 lozenge containing 13.3 mg of zinc (as zinc acetate) or placebo every 2–3 h while awake. The subjective scores for common cold symptoms were recorded daily. Plasma zinc, soluble interleukin (IL)–1 receptor antagonist (sIL-1ra), soluble tumor necrosis factor receptor 1, soluble vascular endothelial cell adhesion molecule, and soluble intercellular adhesion molecule (sICAM)–1 were assayed on days 1 and 5.

Results. Compared with the placebo group, the zinc group had a shorter mean overall duration of cold (4.0 vs. 7.1 days; $P < .0001$) and shorter durations of cough (2.1 vs. 5.0 days; $P < .0001$) and nasal discharge (3.0 vs. 4.5 days, $P = .02$). Blinding of subjects was adequate, and adverse effects were comparable in the 2 groups. Symptom severity scores were decreased significantly in the zinc group. Mean changes in plasma levels of zinc, sIL-1ra, and ICAM-1 differed significantly between groups.

Conclusion. Administration of zinc lozenges was associated with reduced duration and severity of cold symptoms. We related the improvement in cold symptoms to the antioxidant and anti-inflammatory properties of zinc.

Adults and children in the United States experience 2–6 episodes of the common cold per year [1, 2]. The morbidity and loss of working hours due to colds are substantial, and treatment remains unsatisfactory. The complications of the common cold include otitis media, sinusitis, and exacerbations of reactive airway diseases [1–4]. The clinical syndrome of the common cold is caused by a variety of viruses [4]. Rhinoviruses are the most frequent cause and may account for nearly 80% of common colds in autumn [4].

The effect of zinc lozenges on the duration or severity of common cold symptoms has been examined in at least 14 different studies since 1984 [5–16]. In 1984, Eby et al. [5] reported for the first time on the efficacy of zinc gluconate lozenges for treatment of the common cold. Later trials gave inconclusive results [4]. Results of trials in which no effect of zinc was demonstrated were criticized for having inadequate sample sizes or for using inadequate doses of zinc or formulations that reduced the release of zinc ions from the lozenge [17].

It has been hypothesized that there is a direct correlation between reductions in the duration of common cold symptoms and the daily dosage of all positively charged zinc species released from lozenges at physiologic pH [17]. The reanalysis of 10 double-blind, placebo-controlled zinc trials by solution chemistry methods showed a significant correlation between total

Received 12 September 2007; accepted 17 October 2007; electronically published 14 February 2008.

Potential conflicts of interest: none reported.

Financial support: National Institutes of Health (grant 5 R01 A150698–04); George and Patsy Eby Foundation, Austin, Texas (unrestricted research funds to Wayne State University for partial support of this study).

Reprints or correspondence: Dr. Ananda Prasad, Dept. of Internal Medicine, Wayne State University School of Medicine, 1122 Elliman Bldg., 421 E. Canfield, Detroit, MI 48201 (prasada@karmanos.org).

The Journal of Infectious Diseases 2008; 197:795–802

0022-1899/2008/19706-0004$15.00
DOI: 10.1086/528803

daily dosages of positively charged zinc species and a reduction in the mean duration of common colds [18]. Zinc gluconate and zinc acetate have very low chemical stability and mainly release positively charged zinc ions in aqueous solutions at physiologic pH, but stronger complexes do not [18]. Adding a strong zinc-binding ligand, such as glycine or citric acid, to a solution containing a zinc complex that is weakly bonded results in the sequestration of zinc to the stronger ligand, reducing or eliminating the benefits of zinc lozenges [18]. The studies finding a significant effect of zinc lozenges for the treatment of common cold have been criticized for inadequate blinding, either because of poorly matched placebos or because the active preparation was associated with a high incidence of adverse effects [4].

In 2 studies [9, 16], zinc acetate lozenges were associated with a significant reduction in the mean duration of cold symptoms. These studies used compressed lozenges designed by George Eby that were identical in composition. In addition to zinc acetate, they contained directly compressible (agglomerated) dextrose as the tablet base, glycerol monosterate (2.5% of tablet weight) as the tablet lubricant, stevia for added sweetness, and peppermint oil for flavor, with the composition compressed to near-maximal hardness for slowest dissolution. These ingredients were specifically chosen because they do not react with ionic zinc. The slower dissolution of the 4-g lozenges was an advantage over the smaller lozenges in terms of efficacy.

In another study, which involved subjects with experimental rhinovirus colds, zinc gluconate lozenges (13.3 mg of zinc) decreased the median duration of illness in recipients of zinc compared with the placebo group [15]. In subjects with natural cold symptoms, however, the authors observed no effect of either zinc gluconate or zinc acetate on the mean duration of cold symptoms [15]. In this study, however, the study medications (including placebo) were not matched for appearance, flavor, content, and texture [15]. We hypothesize that the beneficial effects of zinc may be due to its antioxidant and anti-inflammatory properties and its effect on intercellular adhesion molecule (ICAM)–1, which is known to be a major cellular receptor for rhinovirus [19, 20].

METHODS

Participants. We recruited 50 volunteers from the Detroit Medical Center (Detroit, Michigan) to participate in a randomized, placebo-controlled trial of the efficacy of zinc acetate lozenges in treating the common cold. Recruitment was started in January 1999 and ended in January 2003. Participants were medical students, house staff, and employees at Wayne State University who were >18 years of age. Participants were informed of the placebo-controlled, double-blind nature of the study, and the study protocol was approved by the Human Investigation Committee of Wayne State University. Each subject was paid $10 for participation and transportation costs. We recruited healthy volunteers who were free of any illness for various laboratory tests as control subjects.

Volunteers were recruited if they had had cold symptoms for 24 h or less and had at least 2 of the following 10 symptoms: cough, headache, hoarseness, muscle ache, nasal drainage, nasal congestion, scratchy throat, sore throat, sneezing, and fever. We excluded persons who were pregnant, had any known immune deficiency disorder or chronic illness, had had symptoms of the common cold for >24 h, or had previously used zinc lozenges to treat the common cold, to assure blinding. In general, subjects were recruited during fall and winter months. Subjects with history of allergies were not excluded.

We chose a sample size of 50 subjects in order to detect a 50% decrease in the mean duration of symptoms, from 8 days in the placebo group to 4 days in the zinc group with a SD of 2 days, using 2-sided $P \leqslant .05$ and an approximate power of 80% or more. In view of our earlier findings, a 50% reduction in the duration of cold symptoms in the treatment group was considered to be a reasonable end point [16].

Intervention. The lozenges were cherry oil–flavored Fast Dry [18] zinc acetate lozenges, manufactured by F & F Foods (Chicago, IL). The active lozenges contained 13.3 mg of zinc as zinc acetate in a hard candy that contained 3.8 g of sucrose and corn syrup and that was prepared using the open-pot batch method, with the active ingredient added last. One hundred percent of the zinc was available at physiologic pH 7.4 in positively charged, ionic form. The placebo lozenges were of identical composition, except that they contained 0.25 mg of sucrose octaacetate rather than the active ingredient, zinc. There were no fats, metal chelators, or other zinc ion–binding agents in either the active or placebo lozenges. The placebo and zinc lozenges were identical in weight, appearance, flavor, and texture and were supplied by George Eby.

A research consultant prepared the randomization code and the packages of medication [21]. The packages were identical in appearance except for the randomization numbers. A research assistant who was blinded to treatment assignments distributed the study medication. Participants were given 50 lozenges and were asked to dissolve 1 lozenge in their mouth every 2–3 h while awake for as long as they had cold symptoms. They were instructed to take no other cold preparations during the study period.

Outcome measures. Our primary end point was the average duration of cold symptoms. Secondary end points were plasma levels of (1) zinc; (2) soluble interleukin (IL)–1 receptor antagonist (sIL-1ra) and soluble tumor necrosis factor (TNF) receptor (sTNF-R) 1; and (3) the plasma adhesion molecules, soluble vascular endothelial cell adhesion molecule (sVCAM)–1 and soluble ICAM (sICAM)–1.

Participants were asked to complete a daily log documenting the severity of symptoms and the medications taken throughout

Table 1. Demographic characteristics of the study participants.

Variable	Zinc group ($n = 25$)	Placebo group ($n = 25$)
Age, mean ± SD (95% CI), years	34.52 ± 14.06 (28.71–40.32)	35.88 ± 13.40 (30.34–41.40)
Sex		
Male	7	9
Female	18	16
Ethnicity		
Black	8	7
White	14	17
Middle Eastern Arab	1	0
Chinese	1	0
Other	1	1
Cigarette smoker		
No	19	16
Yes	6	9
History of allergies		
No	19	21
Yes	6	4

NOTE. Data are no. of participants, unless otherwise indicated. CI, confidence interval.

the duration of the cold. Every day the subjects graded each symptom as 0 for none, 1 for mild, 2 for moderate, or 3 for severe. Total symptom scores were calculated by summing the scores of the 10 symptoms for each day. Resolution of cold symptoms was defined as resolution of all symptoms (a total symptom score of 0) or resolution of all but 1 mild symptom (a total symptom score of 1). The participants were not asked to rate their overall illness or level of severity in order to make their assessments unbiased.

Plasma samples were collected and assayed for levels of zinc, sIL-1ra, sTNF-R1, sVCAM-1, and sICAM-1. Zinc was assayed by using methods established in our laboratory that are based on flameless atomic absorption spectrophotometry [22, 23]. Every precaution was taken to avoid contamination during collection, preparation, and analysis. Variables were analyzed using Quantikine ELISA kits for sIL-1ra, sTNF-R1, sVCAM-1, and sICAM-1 (R&D Systems).

To assess adverse effects of the treatment, participants were given a questionnaire to fill out at the end of the trial. Participants provided yes-or-no answers to questions about nausea, abdominal pain, vomiting, diarrhea, constipation, sweet taste, sour taste, bitter taste, aftertaste, dry mouth, mouth irritation, and bad taste.

Participants returned to the clinic on the fifth day for a blood sampling and again for the final visit within 1 day of resolution of cold symptoms. At this time they returned unused lozenges; lozenges were collected to check adherence and to confirm that cold symptoms had resolved.

Maintenance of blinding. Comparability in taste between zinc and placebo was tested in the participants at the beginning and the end of the trial. The participants filled out a questionnaire in which they were asked to guess whether they had received zinc or placebo lozenges. They had 5 choices: certainly placebo, certainly zinc, do not know, probably placebo, and probably zinc. Subjects who selected certainly or probably and were correct about the type of lozenges they received were considered to be correct. We therefore categorized participants as correct, incorrect, or do not know.

Statistical analysis. We compared the change in outcomes before and after intervention for the zinc and placebo groups. If the changes were normally distributed in both groups, as determined by the Shapiro-Wilk test, we used the unpaired t test to compare mean changes. If the changes were not normally distributed, the differences were examined using the nonparametric Wilcoxon rank-sum test. Multivariate analysis of variance with repeated measures was used to determine the effect of treatment × time on severity scores. Fisher's exact test was used to determine group differences in adverse effects. χ^2 analyses were performed to determine group differences in correctly identifying lozenges at baseline and after treatment. Statistical analyses were completed using JMP IN software (version 5.1.2; SAS Institute) on a MacBook Pro Computer.

RESULTS

Demographics. Table 1 shows the demographic characteristics of the study subjects.

Duration and severity of cold symptoms. Table 2 shows the duration and severity of cold symptoms in the zinc and placebo groups. The average duration of cold symptoms was 4.0 days in the zinc group and 7.1 days in the placebo group ($P < .0001$). The durations of cough, nasal discharge, and muscle ache were significantly shorter in the zinc group than in the placebo group (table 2). In 56% of the zinc-group subjects the

Table 2. Duration of common cold symptoms.

	Duration of cold symptoms, mean ± SD (95% CI), days					
	All subjects			Blinded subjects[b]		
Variable	Zinc group (n = 25)	Placebo group (n = 25)	P[a]	Zinc group (n = 22)	Placebo group (n = 23)	P[a]
Overall symptoms	4.00 ± 1.04 (3.57–4.42)	7.12 ± 1.26 (6.59–7.64)	<.0001	3.54 ± 0.96 (3.11–3.97)	7.39 ± 0.98 (6.96–7.81)	<.0001
Specific symptoms						
Sore throat	1.96 ± 1.83 (1.20–2.71)	3.24 ± 2.93 (2.02–4.45)	.07	2.00 ± 1.90 (1.15–2.84)	3.35 ± 2.98 (2.05–4.63)	.07
Nasal discharge	3.00 ± 1.63 (2.32–3.67)	4.56 ± 3.01 (3.31–5.80)	.02	3.00 ± 1.72 (2.23–3.76)	4.70 ± 3.05 (3.37–6.01)	.02
Nasal congestion	2.20 ± 2.02 (1.36–3.03)	2.56 ± 2.88 (1.36–3.75)	.61	2.18 ± 2.06 (1.26–3.09)	2.43 ± 2.97 (1.15–3.71)	.70
Sneezing	2.64 ± 1.62 (1.96–3.31)	2.60 ± 2.27 (1.66–3.53)	.94	2.59 ± 1.71 (1.83–3.34)	2.74 ± 2.30 (1.72–3.73)	.80
Cough	2.16 ± 1.70 (1.45–2.86)	5.08 ± 2.97 (3.85–6.30)	<.0001	2.14 ± 1.70 (1.38–2.89)	5.35 ± 2.46 (4.07–6.62)	<.0001
Scratchy throat	1.68 ± 1.54 (1.04–2.31)	1.92 ± 2.30 (0.96–2.87)	.66	1.73 ± 1.58 (1.02–2.42)	1.91 ± 2.41 (0.87–2.95)	.76
Hoarseness	1.00 ± 1.44 (0.40–1.59)	2.20 ± 2.90 (1.00–3.39)	.07	1.00 ± 1.45 (0.35–1.64)	2.17 ± 3.02 (0.86–3.48)	.10
Muscle ache	0.80 ± 1.22 (0.29–1.30)	2.00 ± 2.25 (1.06–2.93)	.02	0.82 ± 1.30 (0.24–1.38)	1.70 ± 2.08 (0.70–2.59)	.09
Fever	0.52 ± 1.35 (0.04–1.05)	1.12 ± 2.00 (0.29–1.94)	.22	0.55 ± 1.44 (0.09–1.18)	1.13 ± 2.07 (0.23–2.02)	.27
Headache	1.20 ± 1.32 (0.65–1.74)	1.48 ± 1.71 (0.77–2.18)	.52	1.23 ± 1.38 (0.61–1.83)	1.30 ± 1.66 (0.58–2.02)	.86

NOTE. CI, confidence interval.

[a] Because the data on duration of symptoms were normally distributed according to the Shapiro-Wilk test, a t test was used to analyze the differences between the groups.

[b] Blinded subjects: we excluded 3 in the zinc group and 2 in the placebo group who correctly identified their lozenges as zinc or placebo at the beginning of the study.

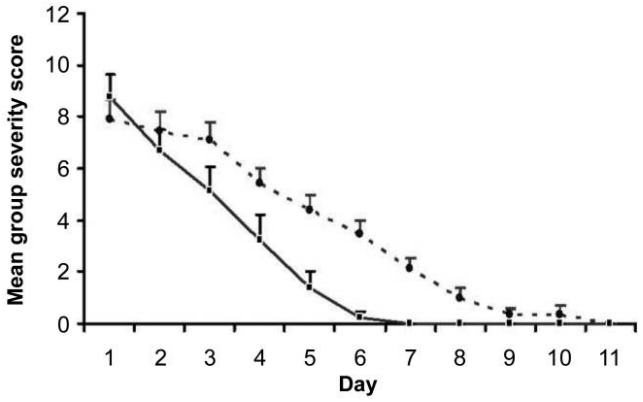

Figure 1. Changes in severity scores after treatment in the zinc *(solid line)* and placebo *(broken line)* groups. Data are given as mean ± SE values. Repeated-measures analysis indicated a significant effect for treatment × time for the zinc group compared with the placebo group over the 10 days of the study ($P = .0002$).

cold was completely resolved on day 4, and no subject in the placebo group was free of cold symptoms on day 4.

The mean overall severity scores for cold symptoms are shown in figure 1. Repeated-measures analysis of severity scores indicated a significant effect for treatment × time for the zinc group compared with the placebo group over 10 days ($P = .0002$). At baseline, the average severity scores for the zinc and placebo groups were 8.32 and 7.78, respectively; by day 4, these scores were 3.45 and 5.61, respectively.

Adequacy of blinding. In the zinc group at the beginning of the study, only 1 subject identified the lozenges as certainly zinc, and 2 subjects identified them as probably zinc. Thus, 3 (12%) of 25 subjects in this group were correct. At the end of the study, 2 (8%) were correct; 1 subject identified the lozenges as certainly zinc, and another subject identified them as probably zinc.

In the placebo group at the beginning of the study, 1 subject said that the lozenges were certainly placebo, and another subject identified them as probably placebo. Thus, 2 subjects (8%) in this group were correct. At the end of the study, none of the subjects identified the placebo lozenge correctly.

Contingency analysis of correctness by group at the beginning of the study yielded $P = 1.0$, by the χ^2 test (Fisher's exact test). At the end of the study, the same test yielded $P = .489$. Thus, there was no significant difference between the 2 groups.

From these data, we concluded that the blinding of the subjects was adequate. In addition, we analyzed the effect of clinical variables for the 22 completely blinded subjects in the zinc group and the 23 in the placebo group; these data are shown in table 2. For completely blinded subjects, cold symptoms lasted for a mean ± SD of 3.54 ± 0.96 days in the zinc group and 7.39 ± 0.98 days in the placebo group ($P < .0001$). The durations of cough and nasal discharge were also significantly shorter in the zinc group (table 2).

Adverse effects. Adverse effects of the zinc and placebo lozenges are compared in table 3. The zinc and placebo groups did not differ significantly in the incidences of any of the adverse effects, including diarrhea, constipation, sweet taste, sour taste, bitter taste, aftertaste, dry mouth, mouth irritation, or bad taste. None of the subjects complained of either abdominal pain or vomiting.

Adherence to therapy was determined by lozenge count. The average number of lozenges taken daily was 6.9 in the zinc group and 6.5 in the placebo group.

Table 4 shows the results of treatment on plasma levels of zinc, sIL-1ra, sTNF-R1, sVCAM-1, and sICAM-1. The plasma zinc level increased significantly in the zinc group. The plasma sIL-1ra level decreased significantly in the zinc group after treatment but increased in the placebo group. A comparison of the mean changes (before vs. after therapy) in plasma sTNF-R1 and sVCAM-1 levels in the 2 groups showed no significant differences between groups. Plasma sICAM-1 levels decreased significantly after treatment in the zinc group.

DISCUSSION

Our results showed that the mean durations of cold symptoms, cough, nasal discharge, and muscle ache were significantly decreased in the zinc group compared with the placebo group. The blinding of therapy was adequate, and our analysis of clinical variables in completely blinded subjects (22 in the zinc group and 23 in the placebo group) showed that the mean durations of cold symptoms, cough, and nasal discharge were also significantly decreased in the zinc group compared with the placebo group in this subset. Zinc treatment was also effective in significantly decreasing the severity score, compared with placebo.

We observed that the mean change in plasma sIL-1ra level showed a significant decrease in the zinc group, whereas in the

Table 3. Adverse effects of zinc and placebo lozenges.

Adverse effect	No. (%) of participants		
	Zinc group ($n = 25$)	Placebo group ($n = 25$)	P[a]
Nausea	3 (12)	1 (4)	.61
Constipation	2 (8)	1 (4)	.22
Diarrhea	1 (4)	1 (4)	1.00
Sweet taste	11 (44)	14 (56)	.57
Sour taste	7 (28)	2 (8)	.13
Bitter taste	8 (32)	12 (48)	.38
Aftertaste	20 (80)	18 (72)	.47
Bad taste	15 (60)	13 (52)	.67
Dry mouth	13 (52)	17 (68)	.37
Mouth irritation	1 (4)	2 (8)	1.00

[a] Fisher's exact test was used to determine group differences.

Table 4. Plasma levels of zinc, anti-inflammatory cytokines, and adhesion molecules.

Variable	Control subjects[a]	Before treatment		After treatment		Mean change		P[b]	Between-group differences in mean changes (95% CI)
		Zinc group	Placebo group	Zinc group	Placebo group	Zinc group	Placebo group		
Zinc, μg/dL	110 ± 10 (31)	96.0 ± 13.6 (25)	101.2 ± 20.5 (25)	111.2 ± 15.5 (25)	99.5 ± 21.1 (25)	15.2 ± 14.8 (25)	−1.7 ± 12.5 (25)	<.0001	16.9 (9.0 to 24.7)
sIL-1ra, pg/mL	293 ± 262 (16)	300 ± 299 (23)	327 ± 186 (24)	264 ± 220 (23)	452 ± 397 (24)	−36.3 ± 182.4 (23)	124.7 ± 304 (24)	.033	−161.1 (−309.1 to –12.2)
sTNF-R1, pg/mL	835 ± 192 (16)	810 ± 300 (24)	883 ± 253 (25)	760 ± 181 (24)	857 ± 297 (25)	−49.3 ± 246 (24)	−25.2 ± 314 (25)	.76	−24.09 (−186.7 to 138.5)
sVCAM-1, pg/mL	492 ± 224 (25)	441 ± 266 (25)	497 ± 257 (24)	492 ± 224 (25)	490 ± 249 (24)	50.9 ± 252 (25)	−6.3 ± 259 (24)	.43	57.3 (−89.6 to 204.2)
sICAM-1, pg/mL	248 ± 82.7 (18)	285 ± 162 (25)	227 ± 149 (24)	229 ± 144 (25)	229 ± 114 (24)	−56.1 ± 95.4 (25)	2.2 ± 103.7 (24)	.04	−58.4 (−115.7 to –1.1)

NOTE. Data are mean ± SD values (no. of subjects), except as indicated. CI, confidence interval; sICAM, soluble intercellular adhesion molecule; sIL-1ra, soluble interleukin-1 receptor antagonist; sTNF-R, soluble tumor necrosis factor receptor; sVCAM, soluble vascular endothelial cell adhesion molecule.

[a] The control group included healthy volunteers who were free of any illness or colds.

[b] P values are for the difference between pre- and posttreatment values for the indicated group. The Shapiro-Wilk test revealed that the distributions of mean differences in the zinc and placebo groups were normal for all tests. Therefore, the variables were analyzed using an unpaired t test.

placebo group the mean change in plasma sIL-1ra level showed a positive increase. The between-group difference in mean change was statistically significant. IL-1ra is anti-inflammatory, functions as a specific inhibitor of IL-1α and IL-1β inflammatory cytokines [24], and is produced by monocytes and macrophages [24]. The synthesis of IL-1ra and IL-1β are differentially regulated at their own promoter sites in macrophages and monocytes [24]. Our results suggest that common cold viruses increase oxidative stress, which activates macrophages and monocytes and result in increased production of both the inflammatory cytokine IL-1β (not assayed here) and the anti-inflammatory product IL-1ra. We also assayed another anti-inflammatory cytokine, sTNF-R1, which functions as a specific inhibitor of TNF activity on target tissues [24]. Levels of sTNF-R1 also decreased in the zinc-treated group, but not significantly. Thus, the decrease in sIL-1ra and sTNF-R1 levels in the zinc group only suggests that zinc decreased the oxidative stress, resulting in decreased activation of monocytes and macrophages. Our previous studies have shown that zinc is an antioxidant [23, 25].

Many of the symptoms observed in the common cold resemble the effects of proinflammatory cytokines [24]. Fever, lack of appetite, leukocytosis, hypoferremia, and induction of acute-phase reactant proteins are known effects of IL-1 production by monocytes and macrophages [24]. We have previously shown that IL-1β production by mononuclear cells is increased in zinc-deficient subjects and is normalized by zinc supplementation, suggesting that zinc modulates the proinflammatory cytokines released by monocytes and macrophages [26].

Figure 2 summarizes our hypothesis as to how zinc may be acting as an antioxidant and anti-inflammatory agent and how it decreases ICAM-1 levels. Infection and oxidative stress activate NF-κB, which increases generation and gene expression of inflammatory cytokines (such as TNF-α, IL-1β, and IL-8) and adhesive molecules (such as VCAM-1 and ICAM-1) [25, 27]. Zinc decreases oxidative stress and induces the zinc-dependent transcription factor A20 in monocytes and macrophages, which inhibits NF-κB activation via the TNF-R–associated factor pathway [25]. Zinc decreases oxidative stress by several mechanisms [23, 25]. Zinc is an inhibitor of NADPH oxidase, an enzyme that initiates the generation of free radicals; it is essential for superoxide dismutase and for generation of metallothionein, a low-molecular-weight protein that removes OH ion effectively (figure 2).

Inflammatory cytokines, generated by activated monocytes and macrophages, are also known to generate greater amounts of reactive oxygen species [27]. Zinc supplementation in healthy human subjects aged 20–50 years reduced the concentration of the oxidative stress markers, such as the oxidative stress–related by-products malondialdehyde, 4-hydroxyalkenals, and 8-hydroxydeoxyguanine in plasma; inhibited the ex vivo induction of TNF-α and IL-1β mRNA in mononuclear cells; and provided protection against TNF-α–induced NF-κB activation in isolated peripheral blood mononuclear cells [25]. We previously provided evidence that, in the human promyelocytic leukemia cell line HL-60, which differentiates to the monocyte and macrophage phenotype in response to phorbol-12-myristate-13-acetate, zinc increases the expression of A20 and the binding of A20 transactivating factor to DNA, which inhibits NF-κB activation [25, 28]. NF-κB is involved in the gene expression of TNF-α and IL-1β in monocytes and macrophages in humans and HL-60 cells, and the effect of zinc in inhibiting the gene expression of TNF-α and IL-1β is cell specific [25, 28]. In a recent study, we showed that, in elderly persons (55–87 years old), ex vivo generation of TNF-α and plasma oxidative stress markers was significantly lower in the zinc-supplemented group than in the placebo group [23]. Given that increased activation of NF-κB in monocytes and macrophages due to viral infection increases the generation and gene expression of adhesive molecules, such as ICAM-1, our observation that only the zinc group showed a significant decrease in plasma ICAM-1 levels suggests that zinc decreased the generation and gene expression of ICAM-1. Human rhinovirus type 14 "docks" with ICAM-1 on the surface of somatic cells [4, 19]. Thus, zinc may in effect act as an antiviral agent by reducing ICAM-1 levels. Another possibility is that zinc ions may form a complex with ICAM-1, preventing the binding of rhinovirus to cells [19].

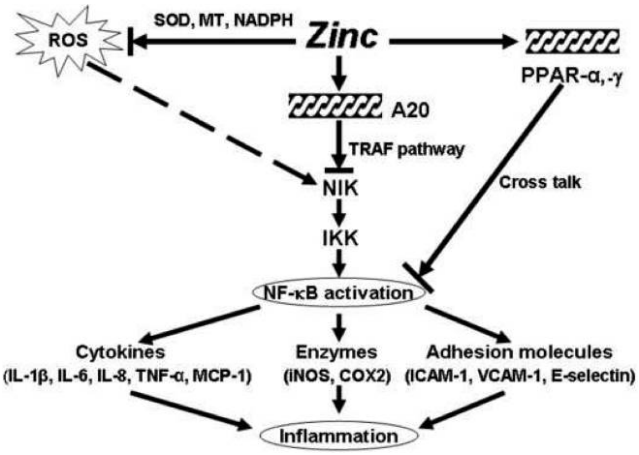

Figure 2. Action of zinc as an antioxidant by various mechanisms. Zinc is essential for superoxide dismutase (SOD) and is an inhibitor of NADPH oxidase. Zinc also induces metallothionein (MT), which is an excellent scavenger for OH ions. Via A20 induction, zinc inhibits NF-κB activation, and this decreases the gene expression and protein generation of various inflammatory cytokines. Decreased activation of NF-κB by zinc also results in decreased gene expression and generation of intercellular adhesion molecule 1 (ICAM-1) (short solid lines indicate blocked activity, and dashed arrow indicates activation). COX, cyclooxygenase; IKK, IκB kinase; IL, interleukin; iNOS, inducible nitric oxide synthase; MCP, macrophage chemoattractant protein; NIK, NF-κB–inducible kinase; PPAR, peroxisome proliferator–activated receptor; ROS, reactive oxygen species; TNF, tumor necrosis factor; TRAF, TNF receptor–associated factor; VCAM, vascular endothelial cell adhesion molecule.

Tremacamra, an sICAM-1 drug that functions as a receptor blockade, has been used to treat experimentally induced rhinovirus infection [20]. This drug was effective in decreasing the severity of common cold symptoms in the experimental model, but it was not clear whether the duration of symptoms was also decreased [20]. In our study, zinc acetate lozenges decreased both the severity and the duration of the common cold. Zinc is less expensive than tremacamra, is relatively nontoxic if ingested in the recommended dosage, and is nonmutagenic. The toxicity and mutagenicity of tremacamra remain to be ascertained.

The ICAM-1 levels at baseline were higher in the zinc group than in the placebo group. Interestingly, the zinc group had more severe cold symptoms than the placebo group at the outset, suggesting that greater severity may have been responsible for higher plasma levels of ICAM-1. More studies are needed to prove this hypothesis.

We conclude that zinc acetate preparation, as used in our study, was significantly effective in decreasing the mean duration of cold symptoms. Our data also show that activation of monocytes and macrophages was decreased by zinc, most likely due to its antioxidant effect. The level of ICAM-1 also showed a significant decrease after zinc therapy, probably due to decreased NF-κB activation by zinc-induced A20 zinc finger protein. We propose that the beneficial clinical effects seen in the zinc group were due to the antioxidant and anti-inflammatory effects of zinc. We also suggest that a decrease in plasma ICAM-1 levels due to zinc therapy may have decreased the docking of the cold viruses on the surface of somatic cells.

References

1. Dingle JH, Badger GF, Jordan WS Jr. Illness in the home: study of 25,000 illnesses in a group of Cleveland families. Cleveland, Ohio: Western Reserve University Press, **1964**.
2. Gwaltney JM Jr, Hendley JO, Simon G, Jordan WS Jr. Rhinovirus infections in an industrial population. I. The occurrence of illness. N Engl J Med **1966**; 275:1261–8.
3. Couch RB. The common cold control? J Infect Dis **1984**; 150:167–73.
4. Turner RB. The treatment of rhinovirus infections: progress and potential. Antiviral Res **2001**; 49:1–4.
5. Eby GA, Davis DR, Halcomb WW. Reduction in duration of common cold by zinc gluconate lozenges in a double-blind study. Antimicrob Agents Chemother **1984**; 25:20–4.
6. Al-Nakib W, Higgins PG, Barrow I, Batstone G, Tryrrell DA. Prophylaxis and treatment of rhinovirus colds with zinc gluconate lozenges. J Antimicrob Chemother **1987**; 20:893–901.
7. Godfrey JC, Conant SB, Smith DS, Turco JH, Mercer N, Godfrey NH. Zinc gluconate and the common cold: a controlled clinical study. J Int Med Res **1992**; 20:234–46.
8. Mossad SB, Macknin ML, Medendorp SV, Mason P. Zinc gluconate lozenges for treating the common cold: a randomized, double-blind, placebo-controlled study. Ann Intern Med **1996**; 125:81–8.
9. Petrus EJ, Lawson KA, Bucci LR, Blum K. Randomized, double-masked, placebo-controlled clinical study of the effectiveness of zinc acetate lozenges on common cold symptoms in allergy-tested subjects. Curr Ther Res **1998**; 59:595–606.
10. Farr BM, Conner EM, Betts RF, Oleske J, Minnefor A, Gwaltney JM Jr. Two randomized controlled trials of zinc gluconate lozenge therapy of experimentally induced rhinovirus cold. Antimicrob Agents Chemother **1987**; 31:1183–7.
11. Douglas RM, Miles HB, Moore BW, Ryan P, Pinnock CB. Failure of effervescent zinc acetate lozenges to alter the course of upper respiratory tract infection of Australian adults. Antimicrob Agents Chemother **1987**; 31:1263–5.
12. Smith DS, Helzner EC, Nuttall CE Jr, et al. Failure of zinc gluconate in treatment of acute upper respiratory tract infections. Antimicrob Agents Chemother **1989**; 33:646–8.
13. Weismann E, Jakobsen JP, Weismann JE, et al. Zinc gluconate lozenges for common cold: a double-blind clinical trial. Dan Med Bull **1990**; 37: 279–81.
14. Macknin ML, Peidmonte M, Calendine C, Janosky J, Wald E. Zinc gluconate lozenges for treating common cold in children. JAMA **1998**; 279: 1962–7.
15. Turner RB, Cetnarowski WE. Effect of treatment with zinc gluconate or zinc acetate on experimental and natural colds. Clin Infect Dis **2000**; 31: 1202–8.
16. Prasad AS, Fitzgerald JT, Bao B, Beck FWJ, Chandrasekar PH. Duration of symptoms and plasma cytokine levels in patients with the common cold treated with zinc acetate. Ann Intern Med **2000**; 133:245–52.
17. Eby GA. Linearity in dose-response from zinc lozenges in treatment of common colds. J Pharm Tech **1995**; 11:110–22.
18. Eby GA. Zinc lozenges: cold cure or candy? Solution chemistry determinations. Bioscience Reports **2004**; 24:23–39.
19. Novick SG, Godfrey JC, Godfrey NH, Wilder HR. How does zinc modify the common cold? Med Hypotheses **1996**; 46:295–302.
20. Turner RB, Wecker MT, Pohl G, et al. Efficacy of tremacamra, a soluble intercellular adhesion molecule 1, for experimental rhinovirus infection: a randomized clinical trial. JAMA **1999**; 281:1797–1804.
21. Begg C, Cho M, Eastwood S, et al. Improving the quality of reporting of randomized controlled trials: the consort statement. JAMA **1996**; 276: 637–9.
22. Prasad AS. Techniques for measurement of zinc in biological samples. In: Biochemistry of zinc. New York: Plenum, **1993**:279–89.
23. Prasad AS, Beck FWJ, Bao B, et al. Zinc supplementation decreases incidence of infections in the elderly: effect of zinc on generation of cytokines and oxidative stress. Am J Clin Nutr **2007**; 85:837–44.
24. Opal SM, De Palo VA. Anti-inflammatory cytokines. Chest **2000**; 117: 1162–72.
25. Prasad AS, Bao B, Beck FWJ, Kucuk O, Sarkar FH. Antioxidant effect of zinc in humans. Free Radic Biol Med **2004**; 37:1182–90.
26. Beck FWJ, Prasad AS, Kaplan J, Fitzgerald JG, Brewer GJ. Changes in cytokine production and T cell subpopulations in experimentally induced zinc-deficient humans. Am J Physiol **1997**; 272:E1002–7.
27. Prasad AS. Zinc: mechanisms of host defense. J Nutr **2007**; 137:1345–9.
28. Bao B, Prasad AS, Beck FWJ, Godmere M. Zinc modulates mRNA levels of cytokines. Am J Physiol Endocrinol Metab **2003**; 285:E1095–102.