Effect of Treatment with Zinc Gluconate or Zinc Acetate on Experimental and Natural Colds

Ronald B. Turner¹ and Wes E. Cetnarowski²

Two clinical trials were conducted, one involving 273 subjects with experimental rhinovirus colds and the other involving 281 subjects with natural colds. Symptomatic volunteers were randomized to receive oral lozenges containing zinc gluconate (13.3 mg), zinc acetate (5 or 11.5 mg), or placebo. The median duration of illness in zinc gluconate recipients was 2.5 days, contrasted with 3.5 days in the placebo recipients ($P = .035$), in the experimental colds study. Zinc gluconate had no effect on symptom severity and zinc acetate had no effect on either duration or severity. Neither formulation had an effect on the duration or severity of natural cold symptoms. Evaluation of blinding, taste, and adverse events revealed no significant differences among the 4 treatment arms. Zinc compounds appear to have little utility for common-cold treatment.

The effect of zinc treatment on the duration or severity of common-cold symptoms has been examined in at least 10 different studies since 1984 [1–9]. In spite of this effort, the effect of zinc on the common cold remains controversial. The inability to definitively answer this question has been attributed to a variety of factors. Studies that have shown no effect of zinc have been criticized as having small sample sizes or for using inadequate doses of zinc or formulations of zinc that might inactivate the zinc salts. On the other hand, the studies that showed a significant effect of zinc have been criticized for inadequate blinding, either because they used poorly matched placebos or because the active preparation was associated with a high incidence of adverse effects.

Recently it has been suggested that the critical determinant of zinc efficacy is the ability of any specific formulation to deliver zinc ions to the oral mucous membranes [10]. On the basis of this analysis, zinc acetate was postulated to be the most active of the zinc salts as a common-cold treatment. The purpose of these studies was to determine the efficacy of zinc acetate for the treatment of common-cold symptoms. The 2 studies, one using the experimental rhinovirus cold model and the other a natural colds model, were designed to specifically address the criticisms of previous clinical trials of zinc treatment.

Materials and Methods

Study medication. Three different preparations were used in these studies: zinc acetate lozenges (5 mg or 11.5 mg; Warner-Lambert, Morris Plains, NJ), zinc gluconate lozenges (13.3 mg; Cold-Eeze, Quigley Corp., Doylestown, PA), and placebo lozenges. The zinc acetate lozenges contained zinc acetate, sugar, glucose syrup, artificial flavoring (citrus), soy lecithin, and artificial coloring. The placebo lozenges contained tannic acid, sucrose octaacetate, sugar, glucose syrup, quinine hydrochloride, artificial flavoring, and artificial coloring.

Although the investigator and the subject were blinded to the identification of the test medications, the study medications were not matched for appearance, flavor, content, and texture. The adequacy of subject blinding was assessed by asking subjects in the challenge study to report whether they were taking active or placebo medication after the first dose of medication and at the end of treatment. In the subset of subjects enrolled at the Medical University of South Carolina (MUSC, Charleston), the taste acceptability of the medication was also assessed at these time points, with use of a 10-cm visual analog scale (from 0 [tastes good] to 10 [tastes bad]). Subjects who met the criteria for randomization to treatment were randomly assigned to 1 of the 4 treatments in accordance with the drug-randomization code. The study medications were dissolved in the mouth and taken every 2–3 waking hours (total of 6 lozenges per day) for up to 14 days.

Induced Colds

Subjects. Subjects were recruited for these studies at MUSC and at Research Testing Laboratories, in Hackensack, NJ. Subjects were required to be in good health and 18–65 years old. In addition, subjects were required to be susceptible to the study virus, as evidenced by a serum neutralizing antibody titer of $\leq 1:4$. Subjects who had a history of allergic disease or nonallergic rhinitis, had abnormal nasal anatomy or mucosa, had had a respiratory tract infection in the previous 2 weeks, were pregnant or lactating, or...
were not taking medically approved birth control were excluded. Subjects were compensated for their participation.

**Challenge virus.** The challenge virus used for this study was rhinovirus type 39. This challenge pool has been safety-tested according to consensus guidelines [11]. All subjects were inoculated with ~100 TCID$_{50}$. The virus was administered as drops in 2 inocula of 250 μL per nostril given ~15 min apart while the subjects were supine.

**Viral isolation, serology, and IL-8 concentrations.** Virus shedding was detected by virus isolation in cell culture, as described previously [12]. Subjects whose viral cultures were positive on any of the post-challenge study days were considered infected. Antibody to the challenge virus was detected by determination of serum neutralizing titers with use of standard methods [13]. The method for measurement of IL-8 concentrations in nasal lavage has been previously described [12, 14].

**Evaluation of illness severity.** Illness severity was assessed by subjective symptom scores. Subjects were asked to judge the maximum severity of 7 symptoms—sneezing, rhinorrhea, nasal obstruction, sore throat, cough, headache, and hoarseness—occurring in the interval since the last symptom evaluation. Each symptom was assigned a severity score of 0–4, corresponding to the reported severity (absent, mild, moderate, severe, or very severe). Before each patient was randomized to treatment, the symptom scores were recorded in an interactive interview with the study staff once each day, and symptom scores for the 7 individual symptoms were summed to yield the total daily symptom score.

After randomization to treatment, the symptom scores were recorded by the subject at ~12-h intervals, just prior to the first and the last daily doses of study medication. The morning and evening symptom scores were averaged to provide a daily symptom score.

**Conduct of the study.** All subjects who met enrollment criteria for the study were challenged with rhinovirus type 39 and then isolated in hotel rooms for the next 5 days. Subjects were randomized to receive study medication 24 h after challenge if they had a total daily symptom score $\geq 3$. Subjects who did not meet this symptom criterion were reassessed 48 h after challenge and randomized if they had a total daily symptom score $\geq 3$. Subjects who did not have a symptom score $\geq 3$ at one of these time points were not randomized to treatment. Treated volunteers continued receiving study medication $\geq 3$ days and until cold symptoms resolved, as defined by 2 consecutive symptom scores $\leq 1$, or until they had received treatment for 14 days.

The duration of the cold was defined as the time from the start of study-medication administration to the first of the 2 consecutive symptom scores $\leq 1$. In the subset of subjects studied at MUSC, nasal lavage was done each morning for the first 5 days after virus challenge, for detection of virus shedding and measurement of IL-8 concentration. The nasal lavage was done after the morning symptom assessment and before the first dose of study medication.

**Natural Colds**

**Subjects.** Volunteers aged 18–65 years with a common-cold illness of recent onset were recruited at 4 different study sites: IMTCI (Lenexa, KS), GFI Pharmaceutical Services (Evansville, IN), TKL Research (Paramus, NJ), and Research Across America (RAA; Dallas).

**Evaluation of illness severity.** Illness severity was assessed by subjective symptom scores, as described for the challenge studies. Before randomization, symptom scores were recorded in an interactive interview with the study staff. After randomization, the symptom scores were recorded by the subject at ~12-h intervals, just before the first and last daily administration of study medication. The morning and evening symptom scores were averaged to provide a daily symptom score.

**Statistical Analyses**

**Sample-size calculation.** The planned sample size in both the experimental and natural cold studies was 64 subjects per treatment arm. This sample size was sufficient to provide 80% power to detect a decrease in the duration of illness from 8 days to 4 days, with $P = .025$ (2-sided).

**Data analysis.** The data were assessed in an intent-to-treat analysis that included all subjects randomized to treatment. The primary efficacy analyses in both studies were the comparisons of the durations of cold symptoms in subjects treated with zinc acetate (5 mg or 11.5 mg) to the durations in those who received placebo. Each of these comparisons was tested at the 0.025 level, for an overall error rate of 0.05. The efficacy of zinc gluconate was tested as a secondary analysis, with $P = .05$ (2-sided) considered significant. Between-group comparisons of the time to cold resolution were performed by means of the log-rank test, adjusted for study site. Kaplan-Meier plots of time to cold resolution were constructed and used to estimate the median time to cold resolution. Before the start of the study a clinically significant decrease in duration of illness was defined as a statistically significant reduction in median duration that was at least 1 day shorter in the treatment group than in the placebo group.

Symptom severity was also analyzed as a secondary end point. The mean total symptom scores (by day and for the first 3 days of treatment) and the mean individual symptom scores (by day and over the first 3 days of treatment) were analyzed with use of pairwise $t$-tests and adjusted means, as well as the pooled error term from an analysis-of-covariance model, with the corresponding baseline score as a covariate and terms for treatment, study site, and treatment-by-site interaction. Consistency across the study sites was assessed by testing the treatment-by-site interaction term at the 0.05 level of significance. Before the start of the study, a clinically significant result was defined as a reduction in symptoms of at least 10% that was also statistically significant. The post hoc
analysis of IL-8 concentration in nasal lavage specimens was done with the Mann-Whitney U test.

Results

Induced Colds

Four-hundred thirteen subjects were challenged with rhinovirus, and 273 (146 at MUSC and 127 at Research Testing Laboratories) met symptom criteria for randomization to study medication. There were no significant differences in the age, sex, race, or height of subjects randomized to the 4 treatment groups. Subjects randomized to the 5-mg zinc acetate group had a significantly lower mean weight (72.3 kg) than the subjects in the other 3 groups (77.7–81.4 kg). The mean total symptom scores (± SEM) at the start of study-medication administration were 5.7 (±0.41) in the placebo, 4.9 (±0.31) in the zinc gluconate group, 5.1 (±0.32) in the 5-mg zinc acetate group, and 5.7 (±0.38) in the 11.5-mg zinc acetate group (P = .263).

Effect of zinc on duration of illness. The median duration of illness was significantly reduced by zinc gluconate (figure 1A). The median duration of illness in zinc gluconate recipients was 2.5 days, in comparison with 3.5 days in the placebo recipients (P = .035). In contrast, zinc acetate had no effect on duration of illness. Colds lasted a median of 3.5 days and 3.25 days in the 5-mg zinc acetate and 11.5-mg zinc acetate groups, respectively.

Effect of zinc on severity of illness. None of the zinc preparations had a significant effect on the severity of common-cold symptoms in the first 3 days of treatment (figure 2A). Analysis of individual symptoms yielded a similar result, with no statistically significant differences in the severity of any of the individual symptoms assessed on any of the 3 days after the start of zinc treatment. The lack of effect on symptom severity was reflected in the absence of an effect on IL-8 concentrations in nasal lavage specimens. Prior to virus challenge the mean IL-8 concentrations in nasal lavage fluid were in the 569 pg/mL in the placebo group, 533 pg/mL in the zinc gluconate group, 585 pg/mL in the 5-mg zinc acetate group, and 529 pg/mL in the 11.5-mg zinc acetate groups. On days 3, 4, and 5 following virus challenge, the mean IL-8 concentrations were 1237 pg/mL in the placebo group, 1336 pg/mL in the zinc gluconate group, 1667 pg/mL in the 5-mg zinc acetate group, and 1475 pg/mL in the 11.5-mg zinc acetate group. There was no significant difference among any of the treatment groups in terms of the IL-8 concentrations on these 3 days, either individually or combined.

Effect of zinc on infection. Although examination of the effect of zinc lozenges on rhinovirus infection was not defined as an outcome measure for this study, a post hoc analysis was done on the subset of subjects enrolled at MUSC. Virus was isolated from 24 (67%) of 36 placebo recipients, from 25 (68%) of 37 zinc gluconate recipients, from 27 (75%) of 36 5-mg zinc acetate recipients, and from 26 (70%) of 37 11.5-mg zinc acetate recipients. These results suggest that zinc treatment had no substantial effect on rhinovirus replication in this study.

Adverse events and adequacy of blinding. Adverse events were reported by 67 (25%) of the placebo recipients, 70 (26%) of the zinc gluconate recipients, 66 (24%) of the 5-mg zinc acetate recipients, and 70 (26%) of the 11.5-mg zinc acetate recipients. The most common adverse events were headache, reported by 39 subjects (14%), and nausea, reported by 13 subjects (5%). After receiving the first dose of study medication, 60% of placebo recipients, 53% of zinc gluconate recipients, 64% of 5-mg zinc acetate recipients, and 66% of 11.5-mg zinc acetate recipients believed they were taking an active medication. At the end of the study, 72%, 64%, 77%, and 79%, respectively, believed they had received an active medication.

Analysis of taste-acceptability in the subset of subjects enrolled at MUSC suggested that unblinding did not occur because of taste. Mean (±SD) visual analog scores at the end of treatment were 1.6 (2.6) in the placebo group, 1.5 (2.6) in the zinc gluconate group, 1.2 (2.1) in the 5-mg zinc acetate group, and 1.5 (2.4) in the 11.5-mg zinc acetate group.

Natural Colds

Two-hundred eighty-one subjects were randomized to receive 1 of the 3 treatments in the natural colds study from November 1997 through February 1998. Thirty subjects were enrolled at IMTCI, 101 at GFI Pharmaceutical Services, 109 at TKL Research, and 41 at Research Across America. No differences were noted in the demographic characteristics of the subjects randomized to the different treatment groups. The mean total symptom scores (±SEM) at the start of study-medication administration were 6.34 (±.39) in the placebo group, 6.7 (±.44) in the zinc gluconate group, 6.3 (±.40) in the 5-mg zinc acetate group, and 6.9 (±.38) in the 11.5-mg zinc acetate group (P = .448).

Effect of zinc on duration of illness. The median duration of illness was not significantly reduced by any of the zinc preparations (figure 1B). The median duration of illness in placebo recipients was 5.5 days, compared with 6.0 days in the zinc gluconate recipients, 6.0 days in the 5-mg zinc acetate recipients, and 5.5 days in the 11.5-mg zinc acetate recipients.

Effect of zinc on severity of illness. None of the zinc preparations had any significant effect on the severity of common-cold symptoms in the first 3 days of treatment (figure 2B). The results were similar for comparisons of the total symptom score over the 3 days or on any of the individual days. Similarly, analysis of individual symptoms revealed no statistically significant differences in the severity of any of the individual symp-
Figure 1. Duration of common-cold symptoms in subjects with induced rhinovirus colds (A) or naturally acquired colds (B) following treatment with zinc gluconate, zinc acetate, or placebo. The median duration of illness in zinc gluconate recipients was 2.5 days, compared with 3.5 days in the placebo recipients (P = .035), in the induced-colds study.

Figures A and B show the percentage of subjects with cold symptoms on each day of treatment over the treatment period. The lines represent different treatment groups: placebo, zinc gluconate, 5-mg zinc acetate, and 11.5-mg zinc acetate.

Adverse events and adequacy of blinding. Adverse events were reported by 7 (10%) of placebo recipients, 11 (16%) of zinc gluconate recipients, 14 (19%) of 5-mg zinc acetate recipients, and 9 (13%) of 11.5-mg zinc acetate recipients. Taste perversion, the most common adverse event, was reported by 6% of placebo recipients, 4% of zinc gluconate recipients, 6% of 5-mg zinc acetate recipients, and 6% of 11.5-mg zinc acetate recipients. Nausea was reported by 7 (2.5%) of the subjects randomized to receive treatment. After receiving the first dose of study medication, 75% of placebo recipients, 70% of zinc gluconate recipients, 58% of 5-mg zinc acetate recipients, and 81% of 11.5-mg zinc acetate recipients believed they were taking an active medication. At the end of the study, 65% of placebo recipients, 60% of zinc gluconate recipients, 44% of 5-mg zinc acetate recipients, and 78% of 11.5-mg zinc acetate recipients believed they were taking an active medication.
mg zinc acetate recipients believed they had received an active medication.

Discussion

Zinc gluconate treatment led to a significant reduction in the median duration of symptoms in volunteers with experimentally induced rhinovirus colds. This observation must be interpreted with caution, however, in light of the fact that zinc gluconate had no effect on the severity of symptoms during the first 3 days of treatment for induced colds and had no effect on either the duration or severity of natural colds. Zinc acetate lozenges had no effect on the duration or severity of symptoms in either the experimental or natural study model.

These studies appear to have addressed many of the criticisms of previous studies of the effect of zinc on common-cold symptoms [15, 16]. Although the placebo treatment was not matched in this study, the responses of the volunteers suggest that the

Figure 2. Effect of treatment with zinc gluconate, zinc acetate, or placebo on mean (± SEM) total symptom score, by day, for subjects with induced rhinovirus colds (A) or naturally acquired colds (B).
study was adequately blinded and that neither unpalatable taste nor the occurrence of adverse events should have affected the results of the study. The sample size in both studies was adequate to detect clinically significant effects of zinc, and both severity of symptoms and duration of symptoms were examined as end points.

The availability of zinc ion has been suggested as a critical determinant of the efficacy of zinc for common-cold treatment, and the lack of zinc ion availability in some formulations has been offered as an explanation of the failure of previous clinical trials to detect a therapeutic effect of zinc [10]. This hypothesis, which predicts that zinc acetate would be an optimal formulation of zinc for common-cold treatment, was not supported by our study.

There are several potential explanations for the disparate results in the experimental and natural models. The more tightly controlled conditions of the induced-cold model reduce variability and may be more sensitive to drug effects [17]. In previous studies in which treatments have been assessed in both the induced-cold and natural-cold models, the drug effect detected in the induced-cold model has been greater than that in the natural model [18, 19]. If the sample size is similar in the 2 models, this increased effect size would result in greater sensitivity to treatment effects. Another potential explanation for the difference in the results in the 2 models is that the effect of zinc gluconate may be specific for rhinovirus infections. The induced-cold model tests the effect of zinc only on rhinovirus colds, although in the natural setting rhinovirus causes only ~50% of all common colds.

The mechanism of any beneficial effect of zinc on common-cold symptoms remains obscure. Although zinc has been found to inhibit rhinovirus replication in vitro, there has been no evidence of an antiviral effect in vivo [3, 4, 20, 21]. The post hoc analysis of cell culture positivity in this study is consistent with these previous findings. It has also been suggested that interaction of zinc with host immune function might have a beneficial effect on common-cold symptoms [22]. The symptoms of the common cold appear to be mediated at least in part by the host response to the viral infection. IL-8, a pro-inflammatory cytokine, is elaborated during rhinovirus colds and has been shown to correlate with symptom severity [12, 23]. In this study there was no detectable effect of zinc on nasal secretion IL-8 concentrations, a finding suggesting that the effects of zinc are not due to modulation of host responses.

In summary, in these studies zinc gluconate had a modest and inconsistent effect on common-cold symptoms, and zinc acetate had no beneficial effect. Furthermore, none of the zinc compounds had an effect on either the ability to recover virus from nasal secretions or the IL-8 response to virus challenge. In light of these results and in the absence of a plausible biological rationale for a therapeutic effect, zinc compounds appear to have little utility for common-cold treatment.

Acknowledgments

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References