Ineffectiveness of Intranasal Zinc Gluconate for Prevention of Experimental Rhinovirus Colds

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Zinc has generally been administered by the oral route in studies of prevention or treatment of the common cold. The purpose of these studies was to evaluate the effectiveness of intranasal zinc gluconate for prevention of experimental rhinovirus infection and illness. Ninety-one volunteers, 41 treated with active medication and 50 treated with placebo, received study medication for 3 days, were inoculated with rhinovirus, and then were treated with study medication for an additional 6 days. Rhinovirus infection was documented in 37 (74%) of the 50 placebo-treated volunteers and in 32 (78%) of the 41 volunteers treated with active medication. Zinc treatment had no effect on total symptom score, rhinorrhea, nasal obstruction, or the proportion of infected volunteers who developed clinical colds. These data do not support a role for intranasal zinc gluconate for prevention or treatment of the common cold.

Since 1984, there have been at least 12 clinical trials conducted to assess the role of zinc in the treatment of the common cold [1–12]. These studies have produced disparate results. The reason for the inconsistency in the outcome of these studies is not clear, but the studies that have failed to find an effect have been criticized for having small sample sizes or using inadequate doses of zinc or formulations of zinc that might inactivate the zinc salts. On the other hand, the studies that have reported a significant effect of zinc have been criticized for inadequate blinding either by the use of poorly matched placebos or because the active preparation was associated with a high incidence of adverse effects.

The rationale for the use of zinc as a common cold therapy is based in large part on the observation that rhinovirus replication is inhibited by zinc [13]. Subsequent study of the antiviral effects of zinc on rhinovirus in vitro revealed that these effects were quite modest and that the therapeutic index was low [14]. Studies of the effect of zinc in vivo have demonstrated no evidence of an antiviral effect on rhinovirus infection [3, 4, 11].

With a single exception [12], studies elsewhere of zinc treatment for the common cold have used oral formulations of zinc salts. Although the salivary concentration of zinc that can be achieved with these formulations is quite high [9], there is no information about the concentration of zinc in the nasal mucosa or in nasal secretions after oral administration of zinc. The site of rhinovirus replication is the nasal mucosa [15, 16], and these infections are more readily initiated by intranasal than intraoral inoculation of virus [17–19]. If zinc has an effect on rhinovirus colds that is mediated by inhibition of rhinovirus replication, it might be expected that this effect would be optimized by direct intranasal administration of zinc. The purpose of these studies was to evaluate the effectiveness of zinc gluconate administered by the intranasal route for prevention of rhinovirus infection and illness.
Subjects. Subjects were recruited for these studies from the university community of the Medical University of South Carolina in Charleston. Subjects were required to be in good health and at least 18 years old. In addition, subjects were required to have a serum neutralizing antibody titer ≥1:4 to the study virus. Subjects with a history of allergic disease or nonallergic rhinitis, abnormal nasal anatomy or mucosa, a respiratory tract infection during the previous 2 weeks, pregnant or lactating women, or women not on medically approved birth control were excluded. Subjects were compensated for participation.

Study medication. Volunteers were randomly assigned to receive either the active preparation or placebo. The active preparation (Zicam) consisted of 33 mM zinc gluconate in an emulsification of benzalkonium chloride, glycerine, hydroxyethylcellulose, sodium chloride, and sodium hydroxide (pH 7.2). The placebo was an identical preparation, with the exception of the omission of the zinc gluconate. Study medications were administered as a single nasal spray of 120 μL per nostril, at ~4-h intervals, 5 times each day. On study days 1 and 2, the first dose of medication was given after the morning nasal lavage. Compliance was assessed by weighing the medication bottles to determine the volume of study medication used and by review of the record of drug administration in the subjects’ diaries. The effectiveness of the placebo blinding was assessed by asking the volunteers to indicate whether they believed they were using the active or placebo preparation just prior to virus challenge.

Challenge virus. The challenge viruses used for this study were rhinovirus type 23 (RV23) and type 39 (RV39). These challenge pools have been safety tested according to consensus guidelines [20]. All subjects were inoculated with a total of 100–300 TCID$_{50}$ per nostril. 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. Virus shedding was detected by virus isolation in cell culture. Nasal wash specimens were collected after symptoms scores were recorded and before the first morning dose of study medication by instillation of 5 mL of 0.9% saline into each nostril. This wash was then expelled into a waxed paper cup and kept chilled until it was processed for viral cultures. Each specimen was inoculated into 2 tubes of human embryonic lung fibroblast cells (MRC-5) and incubated on roller drums at 33°C for 14 days. Rhinovirus was identified by the development of typical cytopathic effect. Subjects with a positive viral culture on any of the postchallenge study days were considered to be infected. Virus titers in the original nasal wash specimens were determined from specimens stored at ~80°C by culturing serial 10-fold dilutions in microtiter plates of MRC-5 cells. For calculation of the mean virus titers, specimens that were positive on initial isolation but negative on reisolation for quantitative culture were defined as containing $10^{0.5}$–$10^{1.2}$ TCID$_{50}$/mL, depending on whether the initial isolate was detected in 1 or both cell culture tubes, and cultures that were negative on initial isolation were defined as containing $10^{-8.5}$ TCID$_{50}$/mL. Serological studies. Antibody to the challenge virus was detected by serum neutralizing titers done by use of standard methods [21]. Serum specimens for antibody testing were collected during screening, immediately prior to virus challenge (acute), and again 21 days later (convalescent). Subjects with at least a 4-fold rise in antibody titer to the challenge virus when the convalescent serum was compared with the acute serum were considered to be infected.

Evaluation of illness severity. Illness severity was assessed by a modification of a method published elsewhere [22, 23]. Symptom scores were recorded prior to virus challenge (baseline) and once each day for the next 5 days. At each evaluation, subjects were asked to judge the maximum severity of 8 symptoms—sneezing, rhinorrhea, nasal obstruction, sore throat,
cough, headache, malaise, and chilliness—in the interval since the last symptom evaluation. Each symptom was assigned a severity score of 0–4, which corresponded to a report of symptom severity of “absent,” “mild,” “moderate,” “severe,” or “very severe.” The daily symptom scores for the 8 individual symptoms were summed to yield the total daily symptom score. The total daily symptom scores for the first 5 days after virus challenge (study days 1–5) were summed to yield the total symptom score. Subjects who had a total symptom score of at least 6 and either at least 3 days of rhinorrhea or the subjective impression that they had a cold were defined as having a clinical cold.

**Study procedures.** All volunteers were treated with study medication for 3 days (days −3 to −1) prior to virus challenge. On the morning of study day 0, volunteers were asked to provide a symptom score and assessment of placebo blinding, a blood specimen was drawn for acute serological examination, a nasal lavage was done for detection of community-acquired viral infections and zinc concentration, and all volunteers were challenged with the study virus. The virus inoculation was done at least 2 h after the first morning dose of study medication. On each of the next 5 days (days 1–5), the volunteers continued use of the study medication and reported to the study site each day for assessment of symptoms. At each of these visits, a nasal lavage was done for viral culture. Approximately 3 weeks after virus challenge, a blood specimen was obtained for determination of the convalescent titer of antibody to the study virus.

**Zinc concentration.** The zinc concentration in nasal lavage fluid was measured by inductively coupled plasma mass spectroscopy. These studies were done in the Trace Elements Laboratory of ARUP Laboratories (Salt Lake City).

**Statistical analysis.** The primary endpoint for this study was the effect of intranasal zinc on the incidence of rhinovirus infection. The effects of zinc on quantitative viral shedding and symptoms were analyzed as secondary outcome variables. All volunteers challenged with virus were included in the analysis of the evaluation of the effect of intranasal zinc on infection. Those subjects who became infected with the study virus were included in the analysis of the effect of intranasal zinc on symptom severity. Proportions were compared with Fisher’s exact test. Symptom scores in zinc and placebo treated subjects were compared with the Mann Whitney U test. P < .05 was considered to be statistically significant.

**RESULTS**

A total of 386 volunteers were screened for the presence of antibody to the challenge viruses; 105 volunteers were enrolled in the study and randomized to study medication. Thirteen subjects either withdrew or were removed from the study prior to the virus challenge (table 1). One subject received placebo medication for part of the study and active zinc medication for the remainder—this subject was considered not evaluable for the endpoints of the study. Ninety-one subjects, 41 randomized to active medication and 50 randomized to placebo, were challenged with virus and completed the study as planned. Thirty-five subjects, 15 treated with active and 20 treated with placebo medication, were challenged with RV39, and 56 subjects, 26 treated with active and 30 treated with placebo medication, were challenged with RV23.

**Compliance with study medications.** Under laboratory conditions, the weight (mean ± SE) of the 90 sprays of study medication administered during the study was 12.4 ± 0.4 g. The actual weight of the spray used was 10.3 ± 0.3 g in the placebo group (83% of predicted) and 9.6 ± 0.4 g in the active treatment group (77% of predicted, P = .28). On the basis of a review of subject diaries, placebo recipients reported missing 0.9 doses, and the recipients of active medication reported missing 1.1 doses over the course of the study. The zinc concentration (mean ± SE) in nasal lavage fluid collected just prior to virus challenge was 47.7 ± 6.1 µg/dL (0.07 mM) in subjects who received active treatment (3 subjects with zinc concentrations of 3354 µg/dL, 971 µg/dL, and 1116 µg/dL were excluded from this calculation) and 6.7 ± 0.5 µg/dL (0.01 mM) in subjects treated with placebo (P < .0001). The measured concentration of zinc in the fluid used for the nasal lavage was 6.5 µg/dL.

**Effect of intranasal zinc on infection.** There was no effect of intranasal zinc treatment on rhinovirus infection. Viral infection with the study virus was documented in 37 (74%) of

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**Table 2. Effect of intranasal zinc gel on illness in volunteers with documented rhinovirus infection.**

<table>
<thead>
<tr>
<th>Evaluation parameter</th>
<th>Placebo medication</th>
<th>Active medication</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>(n = 37)</td>
<td>(n = 32)</td>
</tr>
<tr>
<td>Clinical colds (%)</td>
<td>20 (54)</td>
<td>19 (59)</td>
</tr>
<tr>
<td>Total symptom score (mean ± SE)</td>
<td>11.5 ± 2.4</td>
<td>10.8 ± 1.4</td>
</tr>
<tr>
<td>Total rhinorrhea score (mean ± SE)</td>
<td>2.3 ± 0.58</td>
<td>1.5 ± 0.32</td>
</tr>
<tr>
<td>Total nasal obstruction score (mean ± SE)</td>
<td>2.4 ± 0.50</td>
<td>2.2 ± 0.34</td>
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the 50 placebo-treated volunteers and in 32 (78%) of the 41 volunteers treated with active medication. Infection was documented by virus isolation in 36 (72%) and 30 (73%) of the subjects in the 2 treatment groups, respectively. Infections in the subjects not shedding virus were detected by observation of at least a 4-fold rise in neutralizing antibody to the study virus. The infection rate in volunteers challenged with RV39 (32 [91%] of 35 volunteers) was higher than in those challenged with RV23 (37 [66%] of 56 volunteers; \( P = .01 \)). There was no difference in infection rate between the active and placebo treatment groups with either challenge virus. In those challenged with RV39, the infection rate was 93% (14 of 15) in the volunteers treated with active medication and 90% (18 of 20) in the volunteers who received placebo. Similarly, in the volunteers challenged with RV23, the infection rate was 73% (19 of 26) and 63% (19 of 30) in the active and placebo treatment groups, respectively. Quantitation of virus in nasal lavage of the volunteers challenged with RV39 revealed a significant effect of zinc treatment on the amount of virus shed on days 1 and 2 after virus challenge (figure 1). The quantity of virus shed in volunteers challenged with RV23 was insufficient to permit an assessment of the effect of zinc treatment on quantitative shedding of this virus.

**Effect of intranasal zinc on illness.** There was no significant effect of intranasal zinc treatment on rhinovirus-induced illness. No differences between the active medication– and placebo-treated subjects were noted for the proportion of subjects who developed clinical colds, mean total symptom score, mean rhinorrhea severity score, or mean nasal obstruction severity score (table 2). There were also no significant differences in the mean daily scores for total symptoms, rhinorrhea, or nasal obstruction between the 2 groups (figure 2). Volunteers infected with RV39 had significantly more severe illness than those challenged with RV23. The mean (±SE) total symptom score in RV39-infected volunteers was 19.8 ± 3.8 in placebo-treated subjects and 15.6 ± 2.2 in subjects who received active treatment (\( P = .85 \)). In subjects who were infected with RV23, the total symptom scores were 4.6 ± 1.3 and 7.2 ± 1.4 in the placebo and active medication groups, respectively.

**Adverse events.** There were no serious or frequent adverse events that were judged to be related to the study medication. In the placebo-treated subjects, 21 (42%) reported 32 adverse events. Headache, the most frequent event, was reported by 9 subjects (18%). Adverse events judged to be possibly related to the study medication were nasal tenderness, dry nose, dry mouth, funny taste in mouth, epistaxis (2), nasal burning, and ulcers in mouth. Adverse events in the subjects who received active treatment were similar: 21 subjects (51%) reported 32 different adverse events. Headache, again the most common event, was reported by 10 subjects (24%). Adverse events judged to be possibly related to the study medication were nasal burn-
ing (4 subjects), throat burning (2), throat irritation, dry nose, dry mouth, epistaxis, and nasal tenderness.

Assessment of study blinding. Taste acceptability was assessed after 3 days of treatment and before virus challenge. Six (12%) of the placebo-treated subjects reported that they could taste the study medication, compared with 18 (44%) of the subjects who received active medication ($P = .0008$). Taste acceptability was assessed in those subjects who reported that they could taste the study medication by use of a 10-cm visual analog scale with 0, “taste good” and 10, “taste bad.” The mean ($\pm SE$) taste visual analog score was 5.3 $\pm$ 0.77 in the 6 placebo subjects and 5.5 $\pm$ 0.54 in the 18 subjects on active medication. After 3 days of treatment and prior to virus challenge, 20 (39%) of 51 of the placebo recipients and 21 (51%) of 41 active drug recipients believed they were receiving the active treatment ($P = .29$).

DISCUSSION

The results of this study suggest that prophylaxis with intranasal zinc gluconate has no significant effect on either the incidence or severity of rhinovirus colds. The study was designed to detect a decrease in the infection rate from 90% to 65% (28% decrease), a 45% decrease in total symptom score, or a 35% decrease in the infection rate from 90% to 65% (28% decrease), a 45% decrease in total symptom score, or a 35% decrease in the incidence of Jackson colds with $p_a = 0.05$ (2-sided) and $p_b = 0.2$ (1-sided). A post hoc analysis of the experimental power of the study was done by use of the actual numbers of subjects and the actual infection and illness rates in the placebo-treated group. The study, as conducted, had 80% power to detect a 39% reduction in infection rate, a 68% reduction in total symptom score, or a 52% reduction in clinical colds.

Zinc has been shown to inhibit the replication of rhinovirus, type 39, in vitro at concentrations of 0.03–0.1 mM [14]. However, clinical trials of zinc compounds given as oral lozenges have demonstrated no antiviral activity [3, 4, 11]. In the present study, zinc treatment was associated with significantly lower rhinovirus quantitative titers on the first 2 days after virus challenge, 20 (39%) of 51 of the placebo recipients and 21 (51%) of 41 active drug recipients believed they were receiving the active treatment ($P = .29$). There are substantial differences in the design of the 2 studies. The earlier study addressed the effect of intranasal zinc as treatment for naturally acquired common colds. The time of year when the study was conducted is not reported, so it is not clear whether the fall seasonal peak for rhinovirus infection was included. The effects of zinc on common cold pathogens other than rhinovirus have not been reported. The previous report also required that subjects have at least 3 different symptoms within the first 24 h of illness, a criterion that would select for relatively severe colds. Finally, although adverse events were apparently balanced in that report [12], a formal evaluation of the adequacy of blinding was not reported.

The disparity in the outcomes of different studies of zinc treatment of the common cold remains unexplained. Studies elsewhere that have failed to demonstrate a benefit of zinc treatment have been criticized for using inappropriate formulations of zinc [25]. This criticism is based on a hypothesis that the availability of zinc ion is a critical determinant of the efficacy of zinc for common cold treatment and the presumption that the zinc formulations used in those studies had inadequate “zinc ion availability.” It is important to note that the validity of this hypothesis has not been directly tested. Furthermore, studies that have used identical zinc products have produced dramatically different results [6, 11].

In summary, although suggestive evidence of an antiviral effect of zinc was seen in the present study, this activity was not sufficient to reduce either the incidence of infection or the severity of illness. These data do not support a role for intranasal zinc gluconate for prevention or treatment of the common cold. It seems unlikely that the role of zinc in the common cold will be resolved by additional clinical trials. If additional studies of zinc treatment of the common cold are to be done, they might be more productively directed at testing specific hypotheses for a proposed mechanism of action.

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References


