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Two Randomized Controlled Trials of Zinc Gluconate Lozenge Therapy of Experimentally Induced Rhinovirus Colds

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The therapeutic efficacy of zinc gluconate lozenge therapy in experimentally induced rhinovirus infection was assessed in two randomized controlled trials in susceptible adult volunteers. In trial 1, lozenges containing either zinc gluconate (23 mg of elemental zinc) or placebo were given 36 h after nasal inoculation of rhinovirus type 39 and administered eight times per day for 5 days. All of the volunteers had early cold symptoms at the time that treatment was begun. In trial 2, the same lozenge regimen was used, beginning 2 h after nasal inoculation with rhinovirus type 13, and continued for 7 days. Zinc therapy did not reduce the severity or duration of cold symptoms or the frequency or duration of viral shedding in either trial. Viral titers were measured in trial 2 and were shown to be unaffected by zinc therapy. Nasal mucus weights and the numbers of paper tissues used were slightly higher in zinc recipients. A statistically significant increase in levels of zinc in serum was documented in zinc recipients after 5 days of therapy. These data suggest that zinc gluconate lozenge therapy is not therapeutically useful in the treatment of rhinovirus colds.

The common cold is one of the most frequent afflictions of mankind and the most frequent cause for visits to physicians in the United States. There is still no effective therapy for the many viruses causing this condition. Zinc has been shown to inhibit viral polypeptide cleavage, thus inhibiting the replication of several viruses which cause common colds (1, 2, 9, 10), but the antirhinoviral activity in WI-38 or HeLa cell monolayers is only modest (5). A randomized controlled trial of zinc gluconate lozenge therapy in naturally acquired common colds has suggested remarkable efficacy, with symptom duration reduced by half (4). Blinding efficacy was not assessed in that trial, however, and a higher dropout rate among zinc recipients than among placebo recipients suggests the possibility that blinding was ineffective. We have developed a taste-matched placebo containing denatonium benzoate (a bitter substance used to discourage thumb sucking in children) and have demonstrated in previous studies that our zinc and placebo lozenges are comparable both in palatability and in the proportion of subjects who believe they are receiving active medication (B. M. Farr and J. M. Gwaltney, Jr., J. Chronic Dis., in press). These matched lozenges were used to conduct two placebo-controlled double-blind trials to assess the efficacy of zinc gluconate lozenge therapy in a human model of experimentally induced rhinovirus infection.

MATERIALS AND METHODS

Subjects. Healthy adult volunteers (n = 32) with titers of serum neutralizing antibody to rhinovirus type 39 of ≤1:2 were assigned by prior computer randomization to receive either zinc gluconate or placebo lozenge therapy in trial 1, and 45 healthy adult volunteers with titers of antibody to rhinovirus type 13 of ≤1:2 were enrolled in trial 2. Exclusion criteria included symptoms of any respiratory illness in the week before the study, a history of hayfever, any familiarity with the taste of either denatonium benzoate or zinc, a history of any chronic disease, pregnancy, lactation or an unacceptable contraceptive method in women of childbearing potential, and known abuse of habit-forming drugs. Volunteers gave informed consent for participation in this study after being told that one of the two study lozenges might be an effective therapy for colds. The protocol for trial 1 was approved by the Human Investigation Committee of the University of Virginia, and for trial 2 the protocol was approved by the Institutional Review Board of Essex County, New Jersey.

Lozenges. The lozenges were identical in size, shape, and appearance and were similar in taste, as documented in our previous studies. Both lozenges contained 2% citric acid and a lemon flavoring. The zinc gluconate lozenge (RF 2546; Bristol Myers Products, Hillside, N.J.) contained 23 mg of elemental zinc. The placebo lozenge for trial 1 (RF 2547; Bristol Myers Products) contained 0.00125 mg of denatonium benzoate, and the placebo lozenge for trial 2 (RF 2548; Bristol Myers Products) contained 0.0025 mg of the same compound. Each lozenge was wrapped in cellophane and packaged in an opaque polyethylene bottle bearing the study number, the number of the subject, the treatment day, and dosing instructions.

Viral challenge. Volunteers were admitted to a motel where they were housed in separate rooms from the time of viral challenge until 7 days afterward. Rhinovirus type 39 was administered by intranasal drops (0.25 ml per nostril) on two occasions 30 min apart just after admission to the motel in trial 1. The viral inoculum pool had a titer of $10^{3.5}$ 50% tissue culture infective doses per ml. A 1:10 (vol/vol) dilution of this pool was used for inoculation. Rhinovirus type 13 was similarly administered by intranasal drops (0.25 ml per nostril) from a 1:5,000 (vol/vol) dilution of an inoculum pool with a concentration of $10^{5.8}$ 50% tissue culture infective doses per ml in trial 2.

Treatment plan. Each subject was observed by a nurse when taking each dose of test medication. Subjects were

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TABLE 1. Infection rates and viral shedding in rhinovirus type 39-inoculated volunteers

Type of treatment (no. treated)	No. of subjects shedding virus (%)	No. seroconverting[a] (%)	No. with infection[b] (%)	No. of virus-positive days[c] (%)	Duration of shedding (days)[d]	
					Mean (±SEM)	Median
Zinc (16)	16 (100)	10 (63)	16 (100)	87 (90.6)[e]	6.9 (±0.3)	7
Placebo (16)	14 (88)	9 (56)	15 (94)	66 (73.3)[e]	6.3 (±1.8)	7

[a] Defined by fourfold or greater rise in serum-neutralizing-antibody titer.
[b] Defined by shedding virus or a fourfold or greater rise in serum-neutralizing-antibody titer.
[c] Number of days of observation on which infected subjects shed virus.
[d] Days after viral challenge. Viral cultures were done on study days 2 to 7.
[e] Chi square, 9.52; $P < 0.01$.

instructed not to chew or swallow the lozenges but to allow them to dissolve in the mouth and to raise their hands when only a small residuum remained so that this could be verified and documented on a log sheet for each dose in each subject. Treatment was begun on study day 2 at 8 a.m., 36 h after viral inoculation, in trial 1. In trial 2, treatment was begun 2 h after viral inoculation, which occurred at 7 a.m. on study day 1. The first dose consisted of two lozenges, one followed by the other. Subsequent doses consisted of a single lozenge, and the dosage interval was 2 h. A total of eight doses was administered each day for 5 days in trial 1 and for 7 days in trial 2. The total daily dosages of zinc were 207 mg on day 1 of treatment and 184 mg on each subsequent study day in trial 1 and 184 mg each day in trial 2.

Surveillance and sampling. Trial 1 subjects were interviewed in the afternoon before viral challenge (study day 0) and then each morning on study days 1 through 7 regarding eight common cold symptoms, including sneezing, nasal dischärge, nasal congestion, malaise, headache, chilliness, sore throat, and cough. Subjects were interviewed each morning and afternoon in trial 2. Symptoms were rated for severity according to the following scale: 0, none; 1, mild; 2, moderate; and 3, severe. Criteria for a cold were based on a modification (6) of the criteria proposed by Jackson et al. (8) and required a total symptom score of ≥5 plus either nasal discharge for 3 days or the belief of the subject that a cold had occurred. After discharge from the motel, subjects kept a daily record of the same cold symptoms for 7 days. Each subject was contacted twice by telephone during this period to assure compliance in keeping the diary.

After the study participants blew their noses, their paper tissues were collected and stored in airtight containers. These tissues were collected daily, counted, and weighed to determine nasal mucus weights.

Clinical laboratory tests, including a complete blood count, a differential leukocyte count, a metabolic profile, hepatic enzymes, a urinalysis, and levels of copper and zinc in serum, were obtained on admission to the motel and again on discharge from the motel on study day 7. These tests were all performed with standard methods by Roche Biomedical, Richmond, Va., in trial 1 and by Roche Biomedical, Raritan, N.J., in trial 2.

Infection rates were determined by viral isolation in both trials and /or by documentation of a fourfold or greater rise in serum-neutralizing-antibody titer in paired specimens obtained on the day of viral challenge and 21 days later in trial 1. Nasal wash specimens were collected before viral inoculation and on the morning of each day, beginning with study day 2 and continuing through study day 7. Nasal wash specimens were collected in trial 1 by instilling 2 to 3 ml of 0.85% saline solution into each nostril and then collecting the effluents. This process was repeated until a minimum volume of 4 ml had been obtained, which was mixed with 1 ml of virus-collecting broth. In trial 2, 5 ml of lactated Ringer solution was instilled into each nostril, and the effluent was collected. Viral cultures were grown by standard methods in human embryonic lung cells (WI-38) and MRC5 cells in trial 1 and in MDCK cells and human foreskin fibroblast (HFF) cells in trial 2 (7). Successive 10-fold dilutions of the nasal wash specimens were made and cultured in HFF cell cultures to determine rhinoviral titers of each specimen in trial 2.

Measurement of side effects. Subjects were asked each day about the presence of side effects. On the final day of the study, each subject completed a questionnaire regarding taste, aftertaste, and any adverse effects of the medication, in addition to whether he believed he was given active medication and not placebo. Vital signs were recorded daily.

Statistical analysis. The data were collected manually and entered into a VAX computer, verified by a separate operator, and then checked for logic, consistency, and range before analysis by using an SAS statistical package (11).

Differences in categorical variables were tested by means of the chi-square or Fisher exact test (two-tailed). Continuous variables were compared by using Student's t test. Ordinal variables, such as the cold symptom scale, were analyzed by using the Wilcoxon rank sum test (11).

An alpha level of $P \leq 0.05$ was used for defining the statistical significance of differences between treatment groups. Significant results with the associated probability values are indicated in the tables. For nonsignificant results, probability values are not shown.

The sample size of 32 in trial 1 possessed 72% statistical power to demonstrate the 40% reduction in symptoms reported in the study by Eby et al. (4). Trial 2 possessed 83% power to demonstrate a similar reduction.

RESULTS

Trial 1. In trial 1, 19 (56%) of the subjects (8 zinc, 11 placebo) were women. Of the 32 volunteers, 27 were students (14 zinc, 13 placebo). Only four of the subjects were smokers (2 zinc, 2 placebo). The mean age was 21.4 (± 0.6, standard error of the mean [SEM]) years in the zinc group and 20.6 (±0.5, SEM) years in the placebo group.

The zinc gluconate and placebo groups demonstrated equivalent infection rates of 100 and 94%, respectively (Table 1). All of the infected subjects except for one placebo recipient, who only seroconverted, shed rhinovirus type 39. Only one individual, also in the placebo group, lacked any evidence of infection by viral isolation or seroconversion. The proportions of subjects who seroconverted were similar in the two treatment groups. Infected zinc recipients shed virus more consistently than infected placebo recipients during the study week, but the durations of viral shedding after challenge were not significantly different.

TABLE 2. Illness, symptom scores, and nasal mucus production in rhinovirus type 39-inoculated volunteers

Type of treatment (no. treated)	No. with colds (%)	% with colds of total infected	Nasal symptom score[a]	Total symptom score[a]	Nasal mucus wt (g/5 days)[a]	Paper tissue count (no./5 days)[a]
Zinc (16)	13 (81)	81	9.3 (±1.3)	25.2 (±3.2)	31 (±8)	78.7 (±20.2)
Placebo (16)	12 (75)	80	9.6 (±1.6)	21.5 (±3.8)	27 (±7)	49.9 (±13.7)

[a] Data represent the means (± SEM). These calculations included only infected subjects.

Similar proportions of subjects developed colds in the two groups (Table 2). A breakdown of symptom scores by each study day revealed a significant difference only on day 7, with the zinc recipients having more severe symptoms than placebo recipients. Zinc recipients showed trends toward higher mean mucus weight and a greater number of paper tissues used, but neither trend was statistically significant. Perceptions were not significantly different regarding predominent lozenge taste and palatability or whether medication was active (Table 3).

The frequencies of adverse effects, such as nausea, mouth soreness, and aftertaste, were not significantly different between the two treatment groups. There were no significant differences in vital signs between the two treatment groups. Clinical laboratory tests, including complete blood count, differential leukocyte count, metabolic profile, urinanalysis, and levels of copper and zinc in serum, showed no significant differences between the two groups except for an increased mean level of zinc in the serum of recipients of 105 versus 88 μg of zinc per dl ($P < 0.001$, $t = 4.40$) (normal levels of zinc in serum are 70 to 150 μg/dl in the reference laboratory).

Trial 2. In trial 2, 23 subjects were randomly chosen to receive zinc and 22 were randomly chosen to receive placebo, but one subject in the placebo group was shedding virus on admission to the study and was thus excluded. Another subject was excluded because of a neuromuscular disorder noted after randomization to the placebo group but before viral inoculation. Two more subjects dropped out of the zinc group because of fever and nausea, respectively, on study days 0 and 2. Of the subjects, 41 completed the trial and were available for analysis. There were 13 men in the zinc group (62%) and 9 men in the placebo group (45%). Eight zinc recipients were smokers (38%), compared with six placebo recipients (30%). The mean age was 21.1 (± 0.6, SEM) years in the zinc group and 21.1 (± 0.4, SEM) years in the placebo group.

In each of the two groups (Table 4), 19 subjects were infected. All 38 infected subjects shed rhinovirus type 13. Viral shedding was slightly, but not significantly, more frequent in the infected placebo recipients than in the zinc recipients. Median duration of shedding and geometric mean viral titers were similar for the two groups (Table 5).

In the placebo group, 16 subjects were judged to have a cold, as opposed to 13 in the zinc group. There were no significant differences between the two groups in the frequency, severity, or duration of any individual cold symptoms. There were also no differences in the rate or type of adverse reactions between the two groups.

DISCUSSION

Zinc gluconate lozenge therapy beginning at the onset of cold symptoms had no beneficial effect on experimental rhinovirus type 39 colds in susceptible volunteers in trial 1. Prophylactic postexposure therapy, beginning 2 h after experimental inoculation of rhinovirus type 13, also failed to show any benefits. The proportions of volunteers with colds and the durations of colds were not different, and the severities of cold symptoms were significantly different only during the last day of treatment during trial 1, with zinc recipients having more severe symptoms on that day. The objective measurement of nasal mucus weights was also slightly, but not significantly, greater in the zinc group in both trials. The rate, but not the duration, of viral shedding was significantly increased in the zinc group in trial 1, and there was no difference in shedding titer, frequency, or duration in trial 2.

Zinc gluconate therapy was begun 36 h after viral inoculation in trial 1 and 2 h after inoculation in trial 2 to maximize the chance of detecting therapeutic efficacy. The dosage of zinc gluconate used in this study was selected in an attempt to confirm results of the prior study by Eby et al. that suggested efficacy of this dose in the treatment of natural colds (4). Our formulations of zinc gluconate and the matching placebo lozenges had been previously studied to assure comparability and effective blinding (Farr and Gwaltney, in press).

The subjects in trial 1 showed no significant differences in their perceptions of the two lozenges, and half of the subjects in each treatment group believed they were receiving active treatment.

The failure to demonstrate zinc gluconate efficacy in this study may be interpreted in several ways. It remains possible that the result by Eby et al. (4) was due to an effect against viruses other than rhinoviruses but if so, it would be difficult to explain their dramatic results, given the fact that rhinoviruses constitute the most frequent cause of common colds, especially during the fall, which was the season in which Eby and colleagues conducted their study. It would also be difficult to explain why the zinc gluconate was

TABLE 3. Perception of lozenges by subjects

Type of treatment (no. treated)	No. of subjects (%) noting dominant taste as:					Palatability scale[a]	No. of subjects believing type of treatment was:			No. of subjects noting adverse effects (%)		
	Sweet	Sour	Bitter	Salty	Uncertain		Active	Placebo	Uncertain	Nausea	Sore mouth	Aftertaste
Zinc (16)	7 (43.8)	7 (43.8)	1 (6.3)	0 (0)	1 (6.3)	0.125 (±1.008)	8	8	0	6 (37.5)	8 (50)	5 (31.3)
Placebo (16)	6 (37.5)	6 (37.5)	4 (25)	0 (0)	0 (0)	0.866 (±1.287)	8	7	1	5 (31.3)	8 (50)	2 (12.5)

[a] Palatability scale involved selection of a single number on a +10 (most pleasant) to −10 (most unpleasant) scale; data given are the means (± SEM).

TABLE 4. Infection rates, viral shedding, and illness in volunteers inoculated with rhinovirus type 13

Type of treatment (no. treated)	No. of subjects shedding virus (%)	No. of virus-positive days[a] (%)	Median duration of shedding (days)[b]	No. of subjects with colds (% of subjects shedding virus)	Mean nasal mucus wt (g/7 days)[c]
Zinc (21)	19 (90)	100 (75)	8	13 (68)	16.3
Placebo (20)	19 (95)	113 (85)	8	16 (84)	14.3

[a] Observation days on which viral culture was positive for subjects who shed virus during study.
[b] Median duration of viral shedding in days after challenge. Viral cultures were done on study days 2 to 8.
[c] Mean nasal mucus weights of subjects who shed virus.

TABLE 5. Geometric mean viral titers in nasal wash specimens of volunteers who shed rhinovirus type 13

Treatment	Viral titer[a] (mean ± SEM) on treatment day:						
	2	3	4	5	6	7	8
Zinc	15.9 ± 1.6 (13)[b]	38.0 ± 1.6 (18)	60.3 ± 2.1 (16)	120.2 ± 1.8 (16)	9.6 ± 1.4 (14)	7.8 ± 1.6 (10)	5.6 ± 1.3 (13)
Placebo	14.5 ± 1.4 (13)	79.4 ± 1.6 (19)	47.9 ± 1.8 (19)	53.7 ± 1.7 (19)	20.0 ± 1.5 (16)	11.0 ± 1.4 (14)	11.5 ± 1.5 (13)

[a] 50% Tissue culture infective dose per ml.
[b] Number of subjects with positive cultures for a given study day is given in parentheses.

effective against other viruses but not against rhinoviruses, since previous in vitro data had suggested an antirhinoviral effect (2, 9, 10).

The negative result in this study may be interpreted as having been due to beta error, given the small sample size of the two trials. However, we believe that this is an unlikely explanation of this negative result for several reasons. First, our trials had considerable statistical power (72 and 83%, respectively) to detect a difference in the persistence of symptoms at 5 days of the same magnitude as that reported by Eby et al. (4). Thus, we are fairly confident that a difference this large does not exist for these rhinoviruses. Our study involved the determination of multiple accepted outcome variables, in addition to symptoms (nasal mucus weight, number of paper tissues used, duration of viral shedding, etc.). If zinc were truly effective, the cumulative power of detecting a true difference in at least one of these accepted outcome variables may have been higher than the power calculations cited above. Not only did these two trials fail to show a trend in favor of zinc for any of these outcome variables, but the zinc group actually tended to fare slightly worse than the placebo group in terms of several outcome variables, including the more objective nasal mucus weights and number of tissues used per day.

It is possible that the difference between the results of this study and the results of the study by Eby et al. (4) were due to bias in the latter study. Eby and colleagues used unflavored zinc gluconate tablets and unflavored calcium lactate tablets for lozenge therapy in their trial. Calcium lactate is relatively tasteless and can be easily distinguished on the basis of taste from the rather unpleasant zinc gluconate. Because of a higher rate of side effects and a higher dropout rate in the zinc group in the study of Eby et al., we are concerned that their grossly dissimilar tablets could have resulted in several kinds of bias.

Zinc tablets which taste bad may be more likely to be interpreted as active medication, and subjects believing they are being given active medication may be more likely to give therapeutic credit to zinc tablets even in the absence of true efficacy (Farr and Gwaltney, in press). It is further possible that the noxious side effects of zinc may act in some way similarly to acupuncture in lessening the perception by the subject of the mild, resolving symptoms of a common cold, resulting in colds of shorter duration. It is likewise possible that even if the subject notices the symptoms of the cold, he might be motivated not to report these symptoms so as to stop administration of an unpalatable, nauseating zinc tablet. In trial 1, which involved a more tightly controlled experimental situation with documentation of each dose of medication, 37% of subjects on zinc reported having nausea and 50% reported having a sore mouth.

Recipients of zinc gluconate lozenge therapy did no better and, in fact, did slightly worse in terms of several outcome variables in these two randomized, placebo-controlled, double-blind trials of therapy for experimental rhinovirus infection. We conclude that the previous study suggesting efficacy of zinc gluconate lozenges for treatment of the common cold may have been biased because of the lack of a matching placebo, with resultant unblinding. The problem of bias because of unblinding was noted by Chalmers to account for a trend toward efficacy in a randomized, controlled trial of ascorbic acid treatment of common colds (3). The results of the present study suggest that zinc gluconate should not be regarded as the long-sought cure for the common cold.

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