Prophylaxis and treatment of rhinovirus colds with zinc gluconate lozenges

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Following a tolerance study, double-blind placebo controlled trials were conducted to determine the prophylactic effect of zinc gluconate lozenges on rhinovirus challenge and, in a third study, their therapeutic efficacy when given at the start of colds caused by virus inoculation was tested.

In the prophylaxis study a total of 57 volunteers received lozenges of either zinc gluconate (23 mg) (29 volunteers) or matched placebo (28 volunteers) every 2 h while awake during a period of four and a half days. They were challenged with $10^2$ tissue culture infecting dose (TCID$_{50}$) of human rhinovirus 2 (HRV-2) on the second day of medication, and were monitored daily for symptoms and signs of colds and laboratory evidence of infection. Zinc reduced the total mean clinical score from 8.2 in the placebo group to 5.7 and the reduction of the mean clinical score was statistically significant on the second day after virus challenge.

In the therapeutic study 69 volunteers were inoculated with $10^2$ TCID$_{50}$ of HRV-2 and those who developed cold symptoms were randomly allocated to receive either zinc gluconate lozenges (six volunteers) or matched placebo lozenges (six volunteers) every two hours they were awake for six days. Treatment of colds with zinc reduced the mean daily clinical score and this was statistically significant on the fourth and fifth day of medication. Similarly, medication also reduced the mean daily nasal secretion weight and total tissue count and these reductions were statistically significant on days two and six for nasal secretion weights and days four to six of medication for tissue counts when compared with placebo. There were also statistically significant reductions in the mean total nasal secretion weights and total tissue counts. Zinc, however, had no significant effect on the rate or amount of virus excreted by volunteers.

We conclude that zinc gluconate lozenges are reasonably well tolerated and that they have a significant effect on the signs and symptoms of colds caused by rhinoviruses, although the mechanism of action remains obscure.

Introduction

Zinc, which is a normal and essential constituent of our diet, has been found to inhibit rhinovirus replication in vitro at concentrations of about 0.1 mM (Korant, Kauer & Butterworth, 1974; Butterworth et al., 1976). Eight of nine rhinoviruses studied previously were sensitive to zinc (Korant et al., 1974). However, the mechanism by which zinc displays its antirhinovirus activity is not clear although it has been...
suggested that it may interfere with the cleavage of the rhinovirus polypeptides and hence virion maturation (Butterworth et al., 1976).

Recently Eby, Davis & Halcomb (1984) reported that treatment of patients with common colds with zinc gluconate lozenges significantly improved the course and duration of their illness when compared with those who received placebo. Unfortunately, the Eby et al. (1984) study was not, in our opinion, adequately controlled since it relied on self-assessment by patients, it lacked any laboratory monitoring and the treated and control groups were not well balanced. If substantiated, the results of Eby et al are important and could have wide implications. We have, therefore, conducted double-blind placebo controlled studies to evaluate the efficacy of zinc gluconate lozenges in the prophylaxis and treatment of a rhinovirus infection.

Methods

Volunteers

Healthy volunteers of either sex aged 18 to 50 years participated in three studies, one to determine tolerance to zinc gluconate lozenges, the second to determine the prophylactic effect of zinc gluconate in the prevention of rhinovirus infection and illness and the third to determine the therapeutic efficacy of zinc when symptoms of colds had developed following virus inoculation. The volunteers were housed in isolation in groups of two or three at the MRC Common Cold Unit, Salisbury, in accordance with the Unit's routine practice (Beare & Reed, 1977). They completed a questionnaire for introversion/extroversion and certain obsessional factors since these have been shown to influence the rate of infection and severity of illness (Totman et al., 1980).

Study designs

All volunteers were kept in quarantine for 48 h to ensure they were free of upper respiratory tract symptoms before being allowed to participate in any of the studies. In the tolerance and prophylactic trials, they were divided into groups balanced for age and sex and members of each group were randomly allocated to receive zinc gluconate lozenges or placebo. Thus, ten volunteers (four given zinc and six placebo) participated in the initial tolerance study; further tolerance data were obtained from eight volunteers (three given zinc and five placebo) who were challenged with saline instead of virus to maintain the double-blind nature of the prophylactic study. A total of 57 volunteers took part in the prophylactic study, 29 receiving zinc gluconate lozenges and 28 matched placebo.

Volunteers were asked to suck slowly and dissolve in the mouth one zinc gluconate lozenge containing 23 mg of elemental zinc or placebo every 2 h while they were awake, up to a maximum of 12 lozenges a day. Medication began 24 h before virus challenge and continued for three and a half days afterwards. On the morning of the second day of medication volunteers were challenged with either $10^2$ tissue culture infecting dose (TCID$_{50}$) of human rhinovirus$^2$ (HRV-2) or saline administered in a volume of 1 ml as nose drops (0.5 ml per nostril).

Volunteers in the tolerance study were given the same schedule of medication but were not challenged with virus.
In the therapeutic study, 69 volunteers were inoculated with $10^2 \text{TCID}_{50}$ of HRV-2 and 12 developed colds. These were defined as the use of four or more tissues over the baseline number for that volunteer with at least one other symptom of a cold, e.g. sore throat, nasal stuffiness, sneezing. These 12 volunteers were allocated randomly to receive zinc gluconate lozenges (six volunteers) or matched placebo lozenges (six volunteers) every two hours while they were awake during a period of six days.

Blood was taken for haematological and biochemical profiles on all volunteers before medication and at the end of the trial. In the tolerance and prophylaxis studies a blood sample and a 24 h urine specimen for zinc analysis were collected from each volunteer before medication and again on day three to four after medication.

All trials were double-blind in that neither observers nor volunteers were aware of the allocation of subjects to zinc gluconate or placebo. The studies were approved by the Ethical Committee at Northwick Park Hospital.

**Medication**

Zinc gluconate lozenges (23 mg) and identical placebo lozenges containing no zinc gluconate were supplied by RBS Pharma, Milan, Italy. They were formulated with sugar and flavoured.

**Virus challenge**

A bacteria free pool of nasal washings was prepared from volunteers inoculated with HRV-2, and the washings then diluted in Hanks' saline with 0.2% bovine plasma albumin (BPA). Volunteers received $10^2 \text{TCID}_{50}$ of virus in 1 ml (0.5 per nostril).

**Clinical and laboratory assessment of volunteers**

Volunteers were examined daily and their symptoms and signs recorded and scored. Colds were graded as doubtful, mild, moderate or severe. Nasal secretion weights were measured daily.

Nasal washings were taken before challenge and daily afterwards, and tested for the presence of virus by inoculation into Ohio HeLa cells. The identity of virus isolates was confirmed by neutralization with specific antisera. In the therapeutic studies, virus in the nasal washings was titrated in Ohio HeLa cells to establish the amount excreted by the volunteers each day. Paired sera, the first taken at the beginning of the trial and the second three weeks later, were tested in parallel by virus neutralization tests. Virus isolation and/or four-fold or greater rises in antibody titre were considered to indicate infection.

Serum and 24 h urine zinc levels were assayed by atomic absorption spectrometry using an IL151 spectrophotometer and obtaining a between batch analytical coefficient variation of less than 7%.

**Statistical analysis**

 Frequencies of colds and volunteers shedding virus were compared by a $\chi^2$ test. Scores, nasal secretion weights and virus titres were not normally distributed and so were compared by non-parametric tests. Since the presence of initial neutralizing antibody
can modify the response, a rank analysis of variance was applied with 'blocking' for antibody titre.

Results

Zinc gluconate tolerance study

Ten volunteers participated in the initial tolerance study—four received zinc gluconate and six received placebo. Generally the lozenges were well tolerated despite the relatively large number taken. Some volunteers complained that the taste of food was affected by the lozenges but there were no serious objections to either zinc or placebo lozenges. A similar finding was obtained with the other eight volunteers, of whom three received zinc and five placebo and who were challenged with saline. These results supported those of preliminary tests which indicated that the placebo and medicated lozenges were indistinguishable by appearance and taste. In addition there were no adverse changes in the haematological and biochemical indices.

Prophylactic effect of zinc gluconate lozenges

Table I shows that volunteers were well balanced for age, sex, psychological scores and pre-trial rhinovirus antibody titre. Twenty-nine volunteers received zinc gluconate lozenges and 28 received placebo. They were challenged with $10^2$ TCID$_{50}$ of human rhinovirus type 2 on the second day of medication. Six of the 29 (20%) volunteers who received zinc and eight of 28 (29%) of those on placebo developed significant colds (mild, moderate or severe) (Table I). However, zinc medication reduced the mean daily clinical score on successive days by about one third when compared with placebo and this was statistically significant on the second day after virus challenge (Figure 1). The mean total clinical score was reduced from 8.2 in the placebo group to 5.7 in the zinc group, although this was not statistically significant. Medication with zinc also

| Table I. Prophylaxis with zinc gluconate lozenges: clinical and virological findings |
|---------------------------------|----------------|----------------|----------------|----------------|----------------|
| **Group** | Pre-trial antibody titres | No. of volunteers | No. with significant clinical illness* | Rise in Ab titre | Virus isolated |
|----------|----------------|----------------|----------------|----------------|----------------|
| Drug     | <2            | 15             | 4              | 11             | 12             | 13             |
|          | 2–8           | 12             | 2              | 8              | 10             | 10             |
|          | >8            | 2              | 0              | 0              | 2              | 2              |
| Total    | 29*           | 6 (20%)        | 19 (65.5%)     | 24 (83%)       | 25 (86%)       |
| Placebo  | <2            | 17             | 6              | 12             | 16             | 16             |
|          | 2–8           | 10             | 2              | 5              | 8              | 8              |
|          | >8            | 1              | 0              | 1              | 1              | 1              |
| Total    | 28*           | 8 (29%)        | 18 (64%)       | 25 (89%)       | 25 (89%)       |

*14 Females, 15 males: mean age = 31.55.
*13 Females, 15 males: mean age = 29.39.
*Classed as mild, moderate or severe cold.
Figure 1. Clinical score, nasal secretion weight and virus excretion following rhinovirus challenge of recipients of zinc gluconate (■) or placebo (□). *P < 0.05.
Figure 2. Daily clinical score, nasal secretion weight and concentration of virus excreted following treatment with zinc gluconate (—) or placebo (— —). n = 6; *P < 0.05; **P < 0.01.
Zinc gluconate lozenges and rhinovirus infections

apparently reduced, though not significantly, the mean nasal secretion weight as well as the number of volunteers who excreted virus (Figure 1). None of eight volunteers (three zinc gluconate and five placebo) who where challenged with saline shed virus.

Therapeutic effect of zinc gluconate lozenges

Although there was no marked difference in the incidence of colds in the two groups in the prophylactic study those volunteers receiving zinc did have milder symptoms despite stopping medication before the peak of the illness. We therefore conducted a further study, this time a therapeutic one, in which zinc lozenges were given after symptoms of a cold had developed and were continued for the duration of the trial. Of the 69 volunteers who were inoculated with HRV-2, 12 developed definite symptoms of the common cold on the same day and these were randomly allocated to receive either zinc gluconate lozenges (six volunteers) or matched placebo (six volunteers). As shown in Figure 2, zinc clearly reduced the mean daily clinical scores compared with those of the placebo groups and this was statistically significant on days four ($P < 0.01$) and five ($P < 0.05$) of treatment, The total mean clinical score was also reduced from 41·0 in the placebo group to 27·2 in the zinc treated group. In this study, medication with zinc also reduced substantially the mean daily nasal secretion weights compared with those in the placebo group and the reduction was statistically significant ($P < 0.05$) on days two and six of treatment. The mean total nasal secretion weights were also significantly reduced to 22·0 g in the zinc compared with 51·4 g in the placebo group. In addition, zinc also significantly reduced the mean number of tissues used by volunteers on days four to six after treatment compared with those used by the placebo group (1·42 vs. 2·75, $P < 0.05$; 0·25 vs. 2·17, $P < 0.01$ and 0·33 vs. 1·67, $P < 0.05$, respectively). The total mean tissue count was thus reduced from 21·7 for those who received placebo to 14·3 for those who received zinc; this was statistically highly significant ($P < 0.01$). However, medication with zinc gluconate did not alter the amount of virus excreted in nasal washings, and on day five after treatment the concentration of virus excreted by those who received zinc was higher than that of those who received placebo.

Zinc status and excretion

There was some variation in the concentration of zinc in the plasma of the various volunteers, although no values were below reference limits. Those who had lower concentrations of zinc clearly reached saturation and then excreted zinc in their urine. All volunteers receiving zinc showed a marked increase in urinary zinc excretion. There was a tendency for those in the placebo group who had low concentrations of plasma zinc ($<13 \mu mol/l$) to have higher clinical scores than the rest but this was not statistically significant.

Discussion

Common colds are responsible for a significant proportion of morbidity annually and, as recent statistics from the United States show, cost some 2.5 billion dollars every year in days of work lost and in the sale of proprietary cold remedies and analgesics (Couch, 1984). A remedy or a specific antiviral agent against the common cold
continues to be the goal of many research groups in both the pharmaceutical industry and academic departments. Despite the development of several synthetic molecules, none has yet been found useful in treating colds and only interferons have proved effective in preventing rhinovirus infection and colds (Tyrrell & Al-Nakib, 1987).

Recently, however, Eby et al. (1984) suggested that zinc gluconate lozenges are effective in the treatment of common colds. They showed that medication with zinc reduced significantly the number of days of illness among those who were treated when compared with those given placebo. However, these studies relied on patient's self-assessment of their colds and lacked stringent control and independent clinical observations. Nor were the aetiological agents identified in these patients or the effect of zinc on virus replication monitored. We have, therefore, conducted double-blind placebo controlled trials to assess the efficacy of zinc gluconate lozenges on rhinovirus infection and illness. We chose HRV-2 since our laboratory studies have shown that this serotype was the most sensitive to zinc. We began with a prophylactic study in which zinc medication was started 24 h prior to virus challenge in order to give zinc the best possible chance to exert its effect. The results showed that medication did reduce the mean daily clinical score compared with placebo and this was statistically significant on the second day after virus challenge. Medication also appeared to reduce the mean daily nasal secretion weight as well as the number of volunteers who shed virus but these reductions were not statistically significant compared with placebo. However, as shown in Figure 1, medication ceased just before the peak of the clinical illness in our volunteers (as measured by the mean clinical score and mean nasal secretion weight) and hence it was not clear whether zinc would have had a more significant effect on illness had medication continued longer.

We therefore conducted a therapeutic trial in which volunteers inoculated with $10^2$ TCID$_{50}$ of HRV-2 and who developed symptoms were allocated randomly to receive zinc or placebo. The results show that zinc gluconate lozenges consistently reduced both the mean daily overall symptoms and signs of disease as reflected in the clinical score and the mean nasal secretion weight, the most objective measure of a clinical response. However, zinc had no effect on the rate or amount of virus excreted compared with placebo suggesting that medication may have had an effect on signs and symptoms of the colds rather than on virus replication. If this is the case, it would be interesting to know whether zinc gluconate lozenges would also have the same effect on coronavirus colds or, indeed, on colds caused by other respiratory viruses. It should be noted too that the signs and symptoms were reduced by a third rather than being abolished. Most of our volunteers showed no biochemical indication of zinc deficiency, so the effect is apparently due to temporarily increasing the intake above normal in otherwise healthy subjects.

We would, however, sound a note of caution. An excess intake of zinc, e.g. 150 mg twice a day for six weeks, may result in reduction in lymphocyte stimulation responses as well as significant reduction in the concentration of high density lipoproteins and a slight increase in the level of low density proteins (Chandra, 1984). We therefore recommend that further studies be limited to therapy—in any case volunteers would not tolerate prolonged and repeated use of strongly flavoured lozenges.

We feel that our data warrant large field trials of zinc gluconate lozenges to extend and confirm its efficacy against natural colds in the community. It will also be of interest to study the mechanism of the effect, which is presumably due to alterations in the immune or physiological responses—the concentrations of zinc we found in blood and urine are far below those that are antiviral against the virus used.
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