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# Zinc Salts Inactivate Clinical Isolates of Herpes Simplex Virus In Vitro

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**Using a standard plaque assay and clinical isolates of herpes simplex virus (HSV), we have tested the ability of zinc salts to inactivate HSV. Virus was treated by incubation at 37°C with zinc salts in morpholinepropanesulfonic acid-buffered culture medium and was then diluted and plated onto CV-1 cells for detection and quantitation of remaining infectious virus. Of 10 randomly chosen clinical isolates (five HSV type 1 [HSV-1] isolates and five HSV-2 isolates), seven were inactivated >98% by treatment in vitro with 50 mM zinc gluconate for 2 h and nine were inactivated >97% by treatment with zinc lactate. The effect was concentration dependent. With an HSV-1 isolate, 50 mM zinc gluconate or zinc lactate caused 100% inactivation, 15 mM caused 98 to 99% inactivation, and 5 mM caused 63 to 86% inactivation. With an HSV-2 isolate, 50 and 15 mM zinc gluconate caused 30% inactivation and 5 and 1 mM caused less than 9% inactivation, whereas 50 and 15 mM zinc lactate caused greater than 92% inactivation and 5 and 1 mM caused 37 and 26% inactivation, respectively. The ability of the zinc salts to inactivate HSV was not related to pH in the pH range of 6.1 to 7.6 since inactivation by zinc gluconate or zinc lactate in that pH range was 99.7 to 100% with a 2-h treatment with 50 mM zinc salt. Short (5-min) treatments of selected isolates with zinc gluconate, zinc lactate, zinc acetate, or zinc sulfate yielded inactivation rates of 0 to 55%.**

Zinc is an essential trace element for all eukaryotic organisms. It is required for the catalytic activity or the structural integrity of more than 300 enzymes (1). Zinc-based domains or "zinc fingers" are a hallmark of a wide variety of transcription factors in which proteins that contain these domains interact with DNA in a sequence-specific fashion.

In vitro studies have shown that the presence of low concentrations (0.1 mM) of various zinc salts in a culture medium results in a significant and reproducible antirhinovirus effect (6, 11). The effect of zinc on rhinoviruses is specific since other metal ions have no effect and other picornaviruses are not affected (11). Six placebo-controlled, randomized clinical trials that have tested the effectiveness of zinc against community-acquired common colds have been reported. Although three of these trials (4, 7, 15) showed that zinc ameliorated the symptoms of colds, the others showed that zinc had no benefit (3, 20, 23).

Clinical trials for determination of the effectiveness of zinc in the treatment of herpes simplex virus (HSV) disease have yielded inconsistent or inconclusive results. Tennican et al. (21) used HSV type 2 (HSV-2) (laboratory strain 333) and topical application of zinc sulfate with female mice to demonstrate that daily zinc treatment, initiated 4 h after virus inoculation, provided statistically significant protection against HSV disease compared to that offered by topical application of buffer alone. Topical application of 4% aqueous zinc sulfate was used to treat human subjects with recurrent HSV of less than 48 h in duration (22). Sixteen of 18 subjects (89%) who received treatments with zinc experienced crusting of lesions within 2 days (versus 7 days for subjects who received treatments without zinc) and complete healing in 9.5 days (versus 16 days for subjects who received treatments without zinc). In another study, zinc sulfate-impregnated tampons were tested for their ability to reduce the recurrence of genital HSV infections in 10 women (2). Recurrences occurred within 6 months in three subjects, but the number of subjects was small and there was no placebo group for direct comparison. Other studies (5, 8, 10, 19) have used zinc in conjunction with other agents such that the contribution of zinc to any observed effect was difficult to identify. Thus, the usefulness of zinc salts as a treatment for HSV infection or disease remains unresolved. However, zinc salts, even at very high concentrations, are nontoxic and are potentially useful in the prevention of transmission of any infectious agents that are inactivated in the presence of zinc.

In vitro studies showed that production of laboratory-adapted strains of HSV from infected cells was inhibited by 99% in the presence of 0.1 mM zinc sulfate (13). Partially purified, cell-free HSV-1 (laboratory strain KOS) was completely inactivated by treatment with 15 mM zinc sulfate for 6 h. Electron microscopic studies demonstrated massive deposition of zinc onto the surfaces of HSV virions, and these zinc deposits apparently interfered with the proper functioning of viral glycoproteins, resulting in blockage of the normal mechanism of penetration by membrane fusion (13).

We report here on an investigation into the in vitro effects of various zinc salts on 10 randomly chosen clinical isolates of HSV. Zinc salts were added to cell-free virus at various concentrations and for various lengths of time, and the amount of infectious virus that remained was measured in a plaque assay. Some strains of virus were efficiently inactivated in the presence of 15 to 50 mM zinc salts. The degree of inactivation was dependent on the length of treatment, the type and strain of HSV, and the zinc salt used for the treatment.

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## MATERIALS AND METHODS

**Toxicity.** Since zinc salts are known to be toxic to cells in culture, we first performed experiments to determine the highest level of zinc that could be tolerated by various cell types in a plaque assay. CV-1 (a continuous monkey

TABLE 1. Clinical isolates of HSV tested in this study

| Isolate no. | HSV type | Source | Patient age (yr) | Patient gender |
|---|---|---|---|---|
| 2 | 1 | Esophagus | 58 | Male |
| 3 | 1 | Mouth | 70 | Male |
| 4 | 1 | Genitalia | 3 | Male |
| 14 | 1 | Lip | 22 | Female |
| 18 | 1 | Finger | 3 | Male |
| 1 | 2 | Urethra | 42 | Male |
| 8 | 2 | Vagina | 48 | Female |
| 16 | 2 | Vulva | 51 | Female |
| 19 | 2 | Genitalia | 20 | Female |
| 20 | 2 | Labia | 23 | Female |

kidney cell line), MRC-5 (a continuous human diploid fibroblast cell line), and HNK (human neonatal kidney) cells were maintained in a humidified $CO_2$ incubator by passage and growth in Eagle's minimal essential medium (EMEM; Gibco BRL) with 5% fetal bovine serum and to which penicillin, gentamicin, amphotericin B, and $NaHCO_3$ were added. On the day prior to initiation of toxicity experiments, cells were passed into 24-well plates at a density of 200,000 cells per well. For the toxicity tests, cells were grown in the presence of zinc concentrations in the culture medium ranging from 0 to 400 mM and were monitored visually for 8 days.

Plaque assays for the detection of viable virus following zinc treatment were performed with previously untreated cells at one-half the minimum zinc concentration that caused visible toxicity. Control plaque assays run in the presence of diluted zinc yielded the same plaque counts obtained by assays run with diluted virus in the absence of zinc.

**Clinical isolates.** Randomly chosen isolates of HSV were obtained from the Clinical Virology Laboratory at St. Louis Children's Hospital and Washington University Medical Center. Table 1 gives information on the patients from whom the isolates for this study were derived and the sources of the specimens. Isolates were grown to a 3+ to 4+ cytopathic effect in CV-1 cells, and the cell-free culture medium was frozen in aliquots and was used as the stock virus. One aliquot of each isolate was thawed, and the titer was determined by a plaque assay with CV-1 cells. Other aliquots were thawed as required for the zinc-treatment experiments. Virus titers ranged from $3 \times 10^3$ to $3 \times 10^6$ PFU per ml. In some cases, different titers of the same virus isolate were used in different experiments.

**Zinc solutions.** USP-grade zinc acetate, zinc lactate, and zinc sulfate (all from Sigma Chemical, St. Louis, Mo.) and zinc gluconate (AMEND Chemical, Irvington, N.J.) were used in all experiments. Stock solutions of 250 mM zinc were prepared and sterilized by passage through a 0.22-μm syringe-tip filter. Glycine was present at twice the molar amount of zinc in all zinc gluconate solutions.

**Treatment with zinc salts.** Approximately 10,000 to 50,000 PFU of virus was combined with an appropriate concentration of zinc salt in morpholinepropanesulfonic acid (MOPS)-buffered (50 mM) EMEM growth medium in a total volume of 200 μl, and the mixture was incubated at 37°C with periodic mixing for a specified period of time, depending on the experiment. Mock-infected control tubes without zinc were treated similarly. At the end of the incubation period, the zinc-virus mixture was diluted and the plaque assay was performed as described below.

**Plaque assay.** Samples were diluted with growth medium to remove the toxic effect of zinc on cells and to yield approximately 50 to 250 PFU of virus per 0.25 ml. A 0.25-ml aliquot of diluted virus was plated in triplicate onto CV-1 cells in 12-well tissue culture plates. Virus was allowed to adsorb for 2 h at 37°C, and then the plate was overlaid with 3.0 ml of growth medium (the final zinc concentration in the overlay mixture was thus diluted to <25 μM) as described above, but the medium also contained 0.8% human immune globulin (Gammar; Centeon LLC, Kankakee, Ill.) to prevent secondary plaque formation. The plate was further incubated at 37°C in a humidified $CO_2$ incubator. At 48 h postinoculation, the cells were fixed and stained and the plaques were counted by using a dissecting microscope. The data presented in the tables were calculated from the mean of the three replicate plaque counts for the mock-infected and the zinc-treated samples.

**Statistics.** Statview software was used to analyze data by the Wilcoxon rank sum test (see Table 2) or the paired *t* test (see Table 3).

## RESULTS

**Toxicity of zinc to cultured cells.** Zinc concentrations of 25 to 100 μM were visibly nontoxic to all three cell lines tested, CV-1, HNK, and MRC-5 cells; concentrations of 200 μM and above were visibly toxic to all three cell lines (data not shown).

**Effect of pH on ability of zinc to inactivate HSV.** Since the addition of zinc salts to the growth medium significantly lowered the pH of the solution, we first investigated the effect of pH on the activity of zinc gluconate plus glycine and on the activity of zinc lactate with respect to inactivation of HSV. Experiments were performed in MOPS-buffered growth medium at pH 6.1, 6.6, 7.1, and 7.6. This pH range was chosen because it spans the physiologic pH and it is within the efficient buffering range of MOPS buffer ($pK_a$ = 7.2 at 25°C). Both zinc salts were capable of nearly completely inactivating HSV (>99.7% inactivation) in the pH range of from 6.1 to 7.6 by 2 h of treatment with the zinc salt at 50 mM. All subsequent inactivation experiments were performed in 50 mM MOPS-buffered growth medium at pH 7.1.

TABLE 2. Treatment of HSV isolates with zinc gluconate or zinc lactate[a]

| Treatment and HSV type isolate no.[b] | Mean ± SD PFU | | % Inactivation |
|---|---|---|---|
| | Mock-treated HSV | Zn-treated HSV | |
| Zn gluconate + glycine | | | |
| HSV-1, 2 | 222 ± 28 | 0 | 100 |
| HSV-1, 3 | 150 ± 1 | 1.7 ± 2.8 | 98.9 |
| HSV-1, 4 | 274 ± 19 | 0 | 100 |
| HSV-1, 14 | 220 ± 8 | 0 | 100 |
| HSV-1, 18 | 342 ± 57 | 0 | 100 |
| HSV-2, 1 | 98 ± 17 | 0.3 | 99.7 |
| HSV-2, 8 | 42 ± 8 | 0.3 | 99.3 |
| HSV-2, 16 | 219 ± 23 | 163 ± 24 | 25.5 |
| HSV-2, 19 | 239 ± 43 | 133 ± 23 | 44.4 |
| HSV-2, 20 | 84 ± 9 | 60 ± 2 | 28.6 |
| Zn lactate | | | |
| HSV-1, 2 | 263 ± 23 | 0.7 | 99.8 |
| HSV-1, 3 | 173 ± 3 | 4.3 ± 1 | 97.5 |
| HSV-1, 4 | 321 ± 23 | 0 | 100 |
| HSV-1, 14 | 252 ± 47 | 0 | 100 |
| HSV-1, 18 | 388 ± 61 | 0.3 | 99.9 |
| HSV-2, 1 | 112 ± 10 | 0 | 100 |
| HSV-2, 8 | 37 ± 7 | 6 ± 3 | 83.7 |
| HSV-2, 16 | 322 ± 48 | 6 ± 1 | 98 |
| HSV-2, 19 | 405 ± 26 | 4.3 ± 1 | 98.9 |
| HSV-2, 20 | 91 ± 19 | 0.7 | 99.2 |

[a] Treatment was with 50 mM zinc for 120 min at 37°C. All plaque counts are the averages for three wells.

[b] By the Wilcoxon rank sum test for HSV-1 inactivation by the zinc salt versus HSV-2 inactivation by the same salt, *P* was 0.028 for zinc gluconate plus glycine, and *P* was 0.25 for zinc lactate.

**Treatment of 10 HSV clinical isolates.** Preliminary experiments showed that treatment of clinical isolates of HSV with 50 mM zinc gluconate or zinc lactate for 2 h yielded a high level of inactivation among the few isolates tested (data not shown). Thus, we sought to determine if a 2-h treatment would work effectively against a broader range of clinical isolates. We selected five HSV-1 and five HSV-2 isolates for testing (Table 1). As shown in Table 2, the zinc salts had various activities against these 10 isolates. All five of the HSV-1 isolates were inactivated >97.5% by zinc gluconate or zinc lactate under the conditions used in this experiment. Two of the HSV-2 isolates were inactivated >99% by zinc gluconate, and four were inactivated >98% by zinc lactate. Under the conditions of this experiment, three HSV-2 isolates were somewhat refractory to treatment with zinc gluconate (25 to 44% inactivation) and one was partially refractory to treatment with zinc lactate (83% inactivation). The percent inactivation by zinc gluconate of the five HSV-1 isolates was significantly greater than the percent inactivation of the five HSV-2 isolates (Wilcoxon rank sum test; $P = 0.028$, $z = -2.19$). The percent inactivation by zinc

lactate of five HSV-1 isolates was not significantly different from the percent inactivation of five HSV-2 isolates (Wilcoxon rank sum test; $P = 0.25$, $z = -1.15$).

**Effects of various concentrations of zinc on inactivation of HSV.** In order to demonstrate that the extent of inactivation observed as described above was directly attributable to the presence of zinc salts in the incubation mixture, we performed experiments with various concentrations of zinc. During the 2-h treatment period, HSV-1 isolate 18 was inactivated 100% in the presence of 50 mM zinc gluconate or zinc lactate but only 86 or 63% in the presence of 5 mM zinc gluconate or zinc lactate, respectively. Similarly, HSV-2 isolate 16 was inactivated 29 or 99% by zinc gluconate or zinc lactate, respectively, but only 8 or 37% by 5 mM and only 1.5 or 25% by 1 mM zinc gluconate or zinc lactate, respectively (data not shown). All levels of inactivation by 50 and 15 mM zinc (either gluconate or lactate) were statistically significant. With 5 mM zinc, inactivation of HSV-1 by zinc gluconate ($P = 0.0032$), inactivation of HSV-1 by zinc lactate ($P = 0.015$), and inactivation of HSV-2 by zinc lactate ($P = 0.02$) were statistically significant (compared to the inactivation of mock-treated virus; paired $t$ test), but inactivation of HSV-2 by zinc gluconate was not statistically significant ($P = 0.08$).

**Treatment of HSV clinical isolates with zinc for short times.** Preliminary experiments indicated that with some isolates all times of treatment in the range of 5 min to 2 h resulted in extensive inactivation of the virus. We thus performed experiments to determine the degree of inactivation of representative isolates during a 5-min incubation in the presence of 50 mM zinc salts with three HSV-1 and two HSV-2 isolates in experiment 1 and with two HSV-1 and three HSV-2 isolates in experiment 2. Table 3 shows the results of these experiments.

TABLE 3. Short treatment of HSV isolates with zinc salts[a]

| Expt. no., treatment, and HSV type, isolate no. | Mean ± SD PFU | | % Inactivation |
|---|---|---|---|
| | Mock-treated HSV | Zn-treated HSV | |
| Expt 1 | | | |
| Zn gluconate + glycine | | | |
| HSV-1, 2 | 215 ± 34 | 158 ± 18 | 27 |
| HSV-1, 14 | 107 ± 12 | 70 ± 9 | 35 |
| HSV-1, 18 | 224 ± 28 | 179 ± 13 | 20 |
| HSV-2, 8 | 45 ± 6 | 34 ± 7 | 24 |
| HSV-2, 16 | 159 ± 32 | 99 ± 9 | 38 |
| Zn lactate | | | |
| HSV-1, 2 | 191 ± 29 | 147 ± 22 | 23 |
| HSV-1, 14 | 131 ± 25 | 74 ± 18 | 44 |
| HSV-1, 18 | 180 ± 31 | 151 ± 14 | 16 |
| HSV-2, 8 | 40 ± 7 | 23 ± 5 | 43 |
| HSV-2, 16 | 238 ± 31 | 106 ± 18 | 55 |
| Expt 2 | | | |
| Zn acetate | | | |
| HSV-1, 2 | 303 ± 5 | 234 ± 36 | 23 |
| HSV-1, 18 | 36 ± 5 | 38 ± 6 | −6 |
| HSV-2, 8 | 275 ± 49 | 175 ± 11 | 36 |
| HSV-2, 19 | 234 ± 19 | 145 ± 25 | 38 |
| HSV-2, 20 | 75 ± 9 | 69 ± 8 | 8 |
| Zn sulfate | | | |
| HSV-1, 2 | 295 ± 73 | 151 ± 25 | 49 |
| HSV-1, 18 | 40 ± 8 | 21 ± 1 | 48 |
| HSV-2, 8 | 259 ± 10 | 170 ± 24 | 34 |
| HSV-2, 19 | 213 ± 40 | 140 ± 39 | 34 |
| HSV-2, 20 | 46 ± 4 | 37 ± 5 | 20 |

[a] Treatment was for 5 min at 37°C with 50 mM Zn. All plaque counts are averages for three wells.

## DISCUSSION

The earliest reports on the mechanism of human rhinovirus (HRV) inactivation by zinc provided evidence that the block in replication was at the level of prevention of maturation of capsid proteins. These proteins were presumably altered by the bound zinc and thus could not act as substrates for proteases and were not cleaved. Consequently, they could not be used as structural components of the HRV virion (11, 12). Rossmann et al. (18) determined the crystal structure of HRV-14, which provided the opportunity for virtual visualization of HRV with zinc substituted for the inhibitor 1-[6-(2-chloro-4-methoxyphenoxy)hexyl]imidaxole, which was used in the original crystals (16). The zinc ions fit into a tetrahedral coordination within the canyons on the surface of HRV. In this conformation, the zinc ions line the canyon floor and block access to the floor so that the preferred cell surface binding ligand, intercellular adhesion molecules (ICAM-1), cannot gain access to its docking site on the virus. Thus, the overall effect of zinc on HRV may be twofold: it may block protease activity and it may also block the binding of virions to the cell surface.

The mechanism of inactivation of laboratory strains of HSV is apparently similar to that of HRV. Kümel et al. (13) showed that zinc-inactivated virus cannot properly penetrate a susceptible cell because the bound zinc functionally inactivates the viral surface glycoproteins. Electron micrographs showed deposition of zinc onto the virion surface, which apparently inactivated a required enzymatic function or blocked presentation to the cell of a virion binding site (13). Those investigators demonstrated that growth of virus in the presence of nontoxic concentrations of zinc (0.1 mM) or treatment of free virus in vitro with much higher concentrations of zinc (15 mM) had the same effect on inactivation of the virus.

Our goal in this study was to extend the in vitro investigations of Kümel et al. (13) to include actual clinical isolates of HSV as opposed to laboratory-adapted strains. Their elegant studies demonstrated an effect of zinc on laboratory strains of HSV at the level of penetration. However, the effect was clearly dependent on the type (and perhaps the strain) of HSV being tested, possibly due to differences in the amino acid composition or the extent of glycosylation of glycoproteins B and D on the surfaces of the various strains. Thus, it was of interest to determine if clinical isolates of HSV behaved similarly to the laboratory strains studied by Kümel et al. (13).

Our results of the treatment of 10 clinical isolates with zinc gluconate and zinc lactate confirm and extend the results of Kümel et al. (13) in two ways. First, we showed that zinc is indeed active against a broad range of clinical isolates, and second, we confirmed the observation that various isolates are affected differently by zinc. All five HSV-1 clinical isolates were inactivated >97.5% by both zinc gluconate and zinc lactate. However, the five HSV-2 clinical isolates varied considerably in their susceptibility to zinc. Thus, the variability observed previously among laboratory isolates was also demonstrated among the clinical isolates used in this study.

The inactivation of representative isolates of HSV-1 and HSV-2 by zinc gluconate and zinc lactate was concentration dependent. The HSV-1 isolate used in this experiment (isolate 18) was nearly completely inactivated by 50 and 15 mM zinc gluconate and zinc lactate, but inactivation was only 86 and 63% by zinc gluconate and zinc lactate, respectively, at a concentration of 5 mM. The HSV-2 isolate chosen for this exper-

iment (isolate 16) was less sensitive to zinc gluconate and zinc lactate than the HSV-1 isolate mentioned above (isolate 18). The HSV-2 isolate was inactivated only 30% by 50 or 15 mM zinc gluconate and only 1.5% by 1 mM zinc gluconate. In contrast, this isolate was inactivated >92% by 50 or 15 mM zinc lactate and 25% by 1 mM zinc lactate. It is important to recall that in these experiments we treated about 10,000 to 50,000 PFU of infectious virus and then diluted the treated virus 200-fold in order to detect the remaining infectious virions. Thus, in the experiments in which virtually 100% inactivation was observed, the zinc treatment effectively inactivated 4 $\log_{10}$ of virus in 2 h at 37°C. By comparison, treatment with 15 mM zinc sulfate of the most zinc-sensitive laboratory strain (strain HSV-1 KOS) tested by Kümel et al. (13) resulted in the inactivation of nearly 4 $\log_{10}$ of virus in 3 h at 37°C and 8 $\log_{10}$ of virus in 6 h at 37°C. In our experiments we could never be certain that all virus was inactivated because of the need to dilute the sample in order to remove zinc and eliminate toxicity in the plaque assay. In fact, our actual range of mean plaque counts from all of these experiments was from 36 to 405 PFU. If only one plaque remained in one of the three zinc-treated wells at each extreme (i.e., if the mean of the three counts was 0.3 PFU), this would correspond to 99.2 to 99.93% inactivation of the virus. In other words, the maximum inactivation that we could detect in these assays is between 99.2 and 99.93%.

We were interested to know if HSV could be inactivated by zinc in short time periods and by zinc salts other than the gluconate and lactate salts. We thus tested five representative isolates (three HSV-1 isolates and two HSV-2 isolates in experiments with the gluconate and lactate salts and two HSV-1 isolates and three HSV-2 isolates in experiments with the acetate and sulfate salts) in a 5-min treatment at 37°C with 50 mM zinc as the gluconate (plus glycine), lactate, acetate, or sulfate salt. The results of these experiments were significantly different from those of Kümel et al. (13), but there were some important differences in our experiments. In their time course of inactivation, the highest concentration of zinc sulfate was 15 mM, whereas in our short-treatment experiments we used 50 mM zinc salts. Since the mechanism of zinc inactivation is due to mass-action binding to the virion surface, the rate of binding will increase with increasing concentration. This may have a marked effect on the rate and extent of binding of zinc to the surfaces of the virions and thus on the rate and extent of inactivation by zinc. Finally, the conditions of the incubation of virus with zinc could potentially have a profound effect on the kinetics of inactivation since the amount of free (ionic) zinc is an important factor. Our reactions were performed in MOPS-buffered growth medium at pH 7.1, and their reactions were run in phosphate-buffered saline at pH 7.3. These differences could account for some of the observed discordance in the relatively slow kinetics of inactivation found by Kümel et al. (13) and the more rapid inactivation rate found in the present study.

There is strong evidence that glycoprotein B of HSV is required for virion penetration into cells and also plays a role in pathogenesis (17). However, several other surface glycoproteins are involved in the processes of fusion and penetration (including glycoproteins D, H, L, and K), and any one or all of them might be inactivated or masked by the binding of zinc to the virion surface.

Our interest in the effects of zinc on HSV is related to the reported effects of zinc on HRV. In three clinical trials zinc has been shown to ameliorate the symptoms of the common cold. Unfortunately, none of those trials included virologic studies to show a direct effect of zinc on virus shedding. Given what we know about the mechanism of action of zinc on viruses, it is possible that it will inactivate many viruses with surface glycoproteins that can efficiently bind to zinc ions and that are required for adsorption to or penetration of the host cell. The current study supports this contention. The clinical relevance of this inactivation may depend to a great extent on whether the zinc ions can be delivered to the site of infection at a sufficient concentration to be effective against the virus. In the case of HRV, this may be the cause of the limited success in some clinical trials and a lack of success in others (9, 14). However, in the case of HSV, for which in many situations the zinc could be applied topically to a lesion, the achievable concentration is enormous. In either case, the timing of the treatment is of utmost importance since any significant delay will result in increased pathology which cannot be reversed even by effective treatment.

In conclusion, HSV clinical isolates can be efficiently inactivated by treatment with zinc salts in vitro. The extent of inactivation depends on the strain of HSV, the concentration of zinc, the counterion used, and the length of the treatment.

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