The mechanism of the antiherpetic activity of zinc sulphate

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The molecular mechanism of the effects of zinc ions against herpes simplex virus (HSV) infection was investigated. Zinc sulphate (100 μM) in the culture medium of an HSV-infected African green monkey kidney cell line did not block viral DNA synthesis and, at this concentration, only moderate cytotoxic effects were observed in uninfected cells. Nevertheless, virus yields were reduced to less than 1% of the control. Thus the long standing hypothesis that zinc might block multiplication of HSV by selective intranuclear inhibition of the viral DNA polymerase apparently has lost its validity. Inhibition of virus growth in the absence of severe cytotoxicity must therefore result from other effects of ZnSO₄. Free virus is inactivated by 15 mM-ZnSO₄ within a few hours of its addition. The inactivated virus is defective in the glycoprotein-dependent functions of penetration and, to some extent, adsorption. Electron micrographs show massive deposition of zinc onto virion components. In a virion, transmembrane transport of zinc ions is not expected and the established antiviral effect is therefore explained by an inhibition of virion glycoprotein function after non-specific accumulation of zinc into many virion membrane components.

Introduction

The need for drug safety and the avoidance of development and spread of virus strains resistant to nucleoside analogues (Eggers, 1989) has increased the importance of physiologically tolerable drugs for the treatment of non-severe herpes simplex virus (HSV) infections. Recently, a study on the clinical efficacy of harmless combinations of heparin and ZnSO₄ has been published (Holzmann et al., 1988) but the mechanism of the alleged antiherpetic activity has not been elucidated.

Inhibitory effects of zinc salts on the replication of HSV have been studied in various systems since 1967 (Falke, 1967; Gordon et al., 1975; Shlomai et al., 1975). In cell homogenates, Fridlender et al. (1978) found HSV DNA polymerase to be inhibited by zinc ions at a lower concentration than the cellular DNA polymerases α and β. Despite the conflicting results of other investigators (Gupta & Rapp, 1976), the interpretation of Fridlender et al. (1978) that addition of zinc salts to the culture medium led to a selective inhibition of the HSV DNA polymerase in the nucleus of the infected cell, and thus to a block in HSV replication, found general acceptance. However, accumulation of zinc within the cell is strictly controlled by the homeostatic mechanism and it has recently been demonstrated that zinc uptake into various culture cells is indeed minimal (Meshitsuka et al., 1987).

In order to explain the antiviral effect of zinc, we re-evaluated the effects of ZnSO₄ on HSV replication, adsorption, penetration, viral DNA synthesis and virus egress, and determined the cytotoxicity of zinc ions. We also studied in detail the inactivation of free HSV particles by Zn²⁺. Surprisingly, control experiments of this sort were not part of any of the previously quoted studies on zinc inhibition of HSV. Investigators performing experimental therapy with topical or systemic application of zinc to HSV-infected mice also failed to take into consideration the possibility of direct inactivation of HSV by zinc salts (Tennican et al., 1979).

Methods

Cells and virus. All experiments have been performed on the African green monkey kidney (AGMK) cell line RC 37 Rita (Italldiagnostics) with HSV-1 strains KOS, WAL and ANG and HSV-2 HG52. Cell culture conditions, origin of virus strains, the methods of stock virus preparation at low m.o.i, and the technique of virus titration have been described previously (Glorioso et al., 1984; Kümel et al., 1985; Bishop et al., 1986). The cell culture medium was Eagle's MEM supplemented with 7% foetal calf serum, non-essential amino acids, penicillin and streptomycin. Radioactively labelled virus was prepared by infection of a cell culture (m.o.i. 3) and addition of 20 μCi/ml [5-3H]thymidine to the medium after the adsorption period. After 24 h, cells were lysed by repeated freezing at -70 °C. The virus suspension was subjected to low speed centrifugation (10 min, 1500 g, 4 °C) and dialysed against 10 mM-HEPES, 100 mM-MgSO₄, pH 7.3.
Colony formation. Cells were seeded in appropriate dilutions onto 6 cm plastic dishes. The cell culture fluid was replaced 12 h later by medium containing ZnSO₄. After 48 h the dishes were stained with crystal violet and photographed. The number of single cells, and of colonies containing two, three, four or more cells per colony, was determined.

DNA synthesis in uninfected cells. Culture medium containing ZnSO₄ in various concentrations was added to cell monolayers. At appropriate times the cultures were labelled for 20 min by addition of [3H]thymidine to a concentration of 2 μCi/ml. DNA was extracted 24 h p.i. by sarkosyl lysis and proteinase K digestion, separated from cellular DNA by CsCl centrifugation and characterized by restriction enzyme analysis, as described previously (Kümel et al., 1982).

Effect of ZnSO₄ on virus yield. Cells were infected by HSV-1 at a m.o.i. of 3 p.f.u./cell. At the end of the adsorption period (1 h), non-adsorbed input virus was removed by three cycles of washing with PBS and the medium replaced by a medium containing the appropriate concentration of ZnSO₄. Virus was harvested from the cultures 24 h p.i. by repeated freezing at -70 °C, and titrated.

Virus adsorption kinetics. Virus was radioactively labelled with [3H]thymidine and used to infect cell monolayers in culture dishes (m.o.i. 10) in the presence or absence of ZnSO₄. At different times thereafter non-adsorbed virus was removed by three steps of washing with PBS. The cells were lysed by repeated freezing at -20 °C and radioactivity was determined in TCA-precipitable material.

Effect of ZnSO₄ on virus penetration. Cell monolayers in culture dishes were pre-washed with ice-cold PBS and either mock-infected or infected at an m.o.i. of 0-3 with HSV-1 KOS at 4 °C. After an adsorption period of 2 h, non-adsorbed virus was removed by repeated washing with cold PBS. Medium containing various concentrations of ZnSO₄ was added and the temperature shifted to 37 °C. After 4 h the medium containing ZnSO₄ was replaced by culture medium. Virus yields were determined by titration of cell lysates prepared by freezing at -70 °C 16 h.p.i. One control dish left without zinc treatment was harvested 12 h.p.i. and another 16 h.p.i. If ZnSO₄ prevented penetration, virus yields in ZnSO₄-treated samples should have resembled that from the 12 h controls because penetration would have occurred only after removal of ZnSO₄. If virus penetration occurred in the presence of ZnSO₄, the respective virus yields should correspond with the 16 h control. Mock-infected cultures were infected after temperature shift using the same protocols to demonstrate that zinc treatment had not abolished the ability of cells to adsorb and replicate virus.

Penetration of inactivated virus. Cell cultures in plastic dishes were infected at 4 °C with zinc-inactivated radioactively labelled HSV-1 KOS at an m.o.i. of 10. After 2 h adsorption free input virus was removed by three cycles of washing with ice-cold PBS and the cells were covered with culture medium and shifted to 37 °C. At appropriate times p.i., sample cultures were washed with hypotonic buffer (RSB: 0-01 M-Tris-Cl, 0-001 M-NaCl, 0-0015 M-MgCl₂) and the cells were scraped off the plastic surface and put in Eppendorf tubes. After 10 min swelling on ice the cells were collected by centrifugation (5 min, 500 g, 4 °C) and suspended in RSB containing 2 M-sucrose and 0·5% Triton X-100. Cells were broken at 4 °C by 15 strokes in a glass homogenizer with a tight fitting plunger. The nuclei were collected and cell debris was removed by three cycles of centrifugation (1000 g, 2000 g, 2000 g, 5 min each, 4 °C) and resuspension in RSB/sucrose/Triton X-100. All steps were monitored by phase-contrast microscopy. Radioactivity in acid-precipitable material from the cell nuclei was taken as a measure of viral DNA transported into the cell nucleus after penetration.

Results

Cytotoxicity of ZnSO₄

To establish a meaningful system it was of primary importance to determine the threshold concentration of ZnSO₄ toxic to uninfected cells. Besides microscopic observation, we monitored the kinetics of DNA synthesis and performed colony formation tests in the presence of increasing concentrations of ZnSO₄ in the culture medium (Fig. 1a to e). Colony formation can be considered a sensitive and comprehensive indicator for all metabolic parameters influencing cell growth, DNA synthesis and mitosis. The results obtained were confirmed by a standard dye exclusion test (trypsin blue; Lindl & Bauer, 1987) and amino acid incorporation (data not shown). The applied methods defined a concentration range of continuously increasing cytotoxicity. Zinc at a concentration of 200 μM had a strong influence on cell morphology, DNA synthesis, cell growth and colony formation. At 100 μM, some indications of cytotoxicity could be detected by visual inspection of cell monolayers after 24 h treatment and DNA synthesis and colony formation were impaired to a moderate extent only. At 50 μM, only protein synthesis showed some impairment (confirming the results of Meshitsuka et al., 1987), the other parameters of cytotoxicity remaining virtually unchanged. Thus the upper limit of concentration for determining the influence of ZnSO₄ on HSV replication in the absence of severe cytotoxicity lies between 50 μM and 200 μM.

Effect of ZnSO₄ on HSV replication

This issue was addressed by determining virus yields in the presence of ZnSO₄ (Fig. 2). With a concentration of 50 μM-ZnSO₄ the virus yield after infection with HSV-1 KOS was only slightly reduced, whereas at 100 μM the yield fell sharply to values below 1% of the control. At 200 μM, virus yield was reduced 50-fold. It is remarkable however that at a concentration of ZnSO₄ in which severe cytotoxicity was observed by all the methods applied, cells were still able to maintain some virus synthesis, the virus yield at 300 μM-ZnSO₄ being 8 × 10² p.f.u./ml. The amount of infectious virus in the eclipse phase (2 h.p.i.) was 10 p.f.u./ml whereas the final yield in untreated controls was 3 × 10⁵ p.f.u./ml. This experiment
was repeated with several other virus strains (HSV-1 ANG, HSV-2 HG52) and similar results were obtained (Fig. 2).

**Effects of ZnSO₄ on cellular and viral DNA synthesis**

Because of the earlier suggestion that the primary cause of impairment of virus synthesis by ZnSO₄ was intranuclear inhibition of HSV DNA polymerase, viral and cellular DNA synthesis was studied in the presence of ZnSO₄. Fig. 3 shows the CsCl density gradient profiles for two ZnSO₄ concentrations. The amount of viral DNA synthesis with 100 µM-ZnSO₄ in the culture medium was not distinguishable from the synthesis in the absence of zinc. According to the data on cytotoxicity and virus yield, this concentration of ZnSO₄ is apparently the highest that is compatible with effective synthetic processes in the infected cell. Viral and cellular DNA were unaffected, although formation of infectious virus was very strongly impaired. If the concentration of ZnSO₄ was increased beyond 100 µM, synthesis of both viral and cellular DNA dropped to very low values.

**Effects of ZnSO₄ on virus adsorption, penetration and egress**

In experiments designed to determine virus yield in the presence of ZnSO₄, the compound was added only after virus adsorption. Therefore, the observed reduction in virus yield does not reflect an effect on the adsorption process. A possible influence of ZnSO₄ on HSV adsorption was investigated using [³H]thymidine-labelled HSV-1 ANG. No difference in the absorption kinetics was found whether ZnSO₄ was present or absent (Fig. 4).

Penetration of HSV is a fast process, directly connected to virus adsorption. Hence the influence of ZnSO₄ on penetration was tested by separating the processes of adsorption and penetration by appropriate

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Fig. 1. Cytotoxicity of ZnSO₄ for the AGMK cell line RC 37 Rita. Light microscopy of cell cultures held in culture medium alone (a) or in medium containing ZnSO₄ at either 100 µM (b) or 200 µM (c) for 24 h and examined for morphological changes by phase-contrast light microscopy. Bar markers represent 35 µm. (d) DNA synthesis in uninfected cell. Cultures held in medium containing ZnSO₄ were subjected to a pulse with [³H]thymidine at the times indicated in the figure, washed and the radioactivity was determined in acid-precipitated aliquots of cell homogenates. Without zinc: ●; △, 50 µM-ZnSO₄; ○, 100 µM-ZnSO₄; □, 200 µM-ZnSO₄; ▽, 300 µM-ZnSO₄. (e) Colony formation in the presence of ZnSO₄. Cells were seeded onto culture dishes in appropriate dilution. After 10 h, medium was replaced by unmodified medium, ○; or medium containing ZnSO₄ at 50 µM ○, 100 µM, □, or 200 µM, ■. Two days after seeding, cells were stained with crystal violet and colonies were counted.
temperature shifts (Fuller & Spear, 1985), ZnSO₄ being added during the period of virus penetration. If ZnSO₄ could prevent virus penetration, this would have led to very low titres of virus 16 h after the end of the adsorption period. Table 1 shows the virus yield in cell cultures when ZnSO₄ had been removed after the 4 h period allowed for penetration. Virus yields 16 h after the end of the adsorption period demonstrate that ZnSO₄ does not prevent either penetration or the early events of virus replication.

Since ZnSO₄ is hardly transported into cells (Meshitsuka et al., 1987), one would expect it to exert cytotoxicity by reacting with surface structures of either the cell or the virion itself. Zinc ions might in this way affect the last stage in HSV replication, egress of the virus from the membrane systems of the cell. This possibility was tested by separate determination (by titration) of the ratio of virus remaining within cells to that transported into the supernatant. We compared cell cultures in which HSV-1 had been propagated in the absence or presence of ZnSO₄; the ratio of intracellular to released virus remained the same (data not shown).

Inactivation of free virus by ZnSO₄

The true mechanism underlying the documented antiviral effect of ZnSO₄ (Holzmann et al., 1988) was revealed by experiments where the interaction of ZnSO₄ with free HSV was tested in the absence of cells. Each of three HSV-1 strains (ANG, KOS, WAL) or HSV-2 HG52 was used either in the form of an unpurified stock (Kümel et al., 1982) or a virus purified by sedimentation in a sucrose gradient (Spear & Roizman, 1972). Virus was incubated for various periods of time with ZnSO₄ dissolved in PBS without Ca²⁺ and Mg²⁺ in concentrations ranging from 150 µM to 15 mM. After dialysis or dilution to reduce the zinc concentration the amount of residual virus was determined by titration. Fig. 5 shows
the kinetics of inactivation of HSV-1 KOS by ZnSO₄. The infectivity of free HSV is effectively destroyed by the presence of ZnSO₄.

Zinc inactivation can be demonstrated with purified HSV and the effect is not simulated by a possible pH shift. Incubation with Na₂SO₄ or MgSO₄ does not inactivate HSV, whereas zinc salts other than the sulphate show an effect similar to that of ZnSO₄ (Table 2).

Inactivation is strongly temperature-dependent (Fig. 5). Even at high concentration (15 mM), ZnSO₄ had no significant effect on HSV infectivity in the temperature range 4 °C to 18 °C. With elevation of the temperature above 20 to 25 °C, inactivation increased strongly. We observed considerable differences in the dose dependence of ZnSO₄ inactivation between individual HSV strains (Fig. 6).

The consequence of inactivation might be impairment of the main glycoprotein functions, adsorption and penetration. Alternatively, virus might be precipitated, aggregated or have its structure destroyed by the divalent cation. We used [³H]thymidine-labelled HSV-1 KOS to study glycoprotein functions of zinc-inactivated virus. The kinetics of virus adsorption was determined for native or inactivated virus (Fig. 7). In a parallel experiment, each of the two virus preparations was adsorbed to cells and the appearance of acid-precipitable radioactive material in cell nuclei was followed (Fig. 8).

We found that DNA of native virus accumulated in nuclei in a linear fashion, whereas no DNA from inactivated virus could be detected from 0 to 4 h p.i. These results suggest that zinc-inactivated virus is not blocked in adsorption but penetration or transport to cell nuclei is apparently totally prevented.

In order to test for morphological alterations or aggregation of the virus we used transmission electron microscopy (Fig. 9). In electron microscopy, biological structures are visualized by the adsorption of an electron-dense heavy metal so zinc adsorption into the virion can be monitored if uranyl acetate contrasting is avoided.

Table 1. Influence of ZnSO₄ on HSV penetration

<table>
<thead>
<tr>
<th>Infection</th>
<th>ZnSO₄ concentration (μM) allowed during virus penetration</th>
<th>Interval allowed for virus replication (h)</th>
<th>Virus yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV-1 KOS</td>
<td>0</td>
<td>12</td>
<td>2.2 x 10^3</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>16</td>
<td>1.0 x 10^7</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>16</td>
<td>2.2 x 10^3</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>16</td>
<td>1.8 x 10^7</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>16</td>
<td>1.9 x 10^7</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>16</td>
<td>1.5 x 10^7</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>16</td>
<td>2.8 x 10^7</td>
</tr>
<tr>
<td>Mock</td>
<td>0</td>
<td>16</td>
<td>2.5 x 10^3</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>16</td>
<td>2.8 x 10^3</td>
</tr>
</tbody>
</table>

Fig. 5. Inactivation of free HSV-1 KOS by ZnSO₄; kinetics and temperature dependence. Free virions of HSV-1 KOS were incubated in the absence of cells with various concentrations of ZnSO₄. The residual virus concentration was determined by titration. □, Incubation at 30 °C, 15 mM-ZnSO₄; ○, incubation at 20 °C, 15 mM-ZnSO₄; △, incubation at 37 °C: △, no zinc; •, 0.5 mM-ZnSO₄; □, 1.5 mM-ZnSO₄; △, 5 mM-ZnSO₄; △, 15 mM-ZnSO₄.

Table 2. Influence of counter ions and pH on the zinc inactivation of the HSV-1 virion

<table>
<thead>
<tr>
<th>Virus preparation*</th>
<th>Inactivation</th>
<th>Residual titre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purified virus†</td>
<td>PBS pH 4.5</td>
<td>1.5 x 10⁸</td>
</tr>
<tr>
<td></td>
<td>PBS pH 5.5</td>
<td>2.0 x 10⁸</td>
</tr>
<tr>
<td></td>
<td>PBS pH 6.5</td>
<td>3.5 x 10⁸</td>
</tr>
<tr>
<td></td>
<td>PBS pH 7.3</td>
<td>3.7 x 10⁸</td>
</tr>
<tr>
<td>Stock virus‡</td>
<td>PBS (pH 7.3)</td>
<td>4.2 x 10⁸</td>
</tr>
<tr>
<td></td>
<td>5 mM-ZnSO₄</td>
<td>5.1 x 10⁸</td>
</tr>
<tr>
<td></td>
<td>5 mM-Zn(CH₃COO)₂</td>
<td>4.1 x 10⁸</td>
</tr>
<tr>
<td></td>
<td>5 mM-Zn(NO₃)₂</td>
<td>3.8 x 10⁸</td>
</tr>
<tr>
<td></td>
<td>5 mM-ZnCl₂</td>
<td>2.5 x 10⁸</td>
</tr>
<tr>
<td></td>
<td>5 mM-ZnI₂</td>
<td>2.5 x 10⁸</td>
</tr>
<tr>
<td></td>
<td>5 mM-Na₂SO₄</td>
<td>3.7 x 10⁸</td>
</tr>
<tr>
<td>Stock virus§</td>
<td>PBS pH 7.3</td>
<td>1.4 x 10⁸</td>
</tr>
<tr>
<td></td>
<td>5 mM-ZnSO₄</td>
<td>2.0 x 10⁸</td>
</tr>
</tbody>
</table>

* Various preparations of HSV-1 KOS were incubated with equal amounts of PBS or salts dissolved in PBS pH 7.3 for 24 h at 37 °C and the residual virus concentration was determined by titration.
† Dialysed against 0.9% NaCl, 10 mM-Tris-HCl pH 7.3.
‡ Grown in medium without protein supplements, dialysed against 100 mM-MgSO₄, 10 mM-HEPES pH 7.3.
§ Virus suspension in unmodified culture medium.

Purified virus (HSV-1 ANG) was inactivated (15 mM-ZnSO₄, 24 h, 37 °C) or, as a control, incubated under the same conditions with buffer only. Both samples were subjected to preparation for electron microscopy; the
Fig. 6. Inactivation of free HSV-1 KOS virions by ZnSO₄; dose dependence and strain differences. Suspensions of HSV-1 KOS (□), HSV-1 ANG (○), HSV-1 WAL (●) and HSV-2 HG52 (△) were incubated for 24 h with ZnSO₄ at the concentrations indicated and the residual virus concentration was determined.

Fig. 7. Adsorption kinetics of ZnSO₄-inactivated virus. Radioactively labelled HSV-1 KOS was inactivated in 15 mM-ZnSO₄ for 24 h or incubated with PBS as a control. Virus adsorption was monitored by removal of unadsorbed virus and determination of radioactivity in acid-precipitable material at the times indicated (for details see Methods). Native virus, △; inactivated virus, ●.

Discussion

The mechanism of inhibition of HSV infection by ZnSO₄ has had a chequered history in the literature. An early study (Falke, 1967) described alteration of giant cell formation, a glycoprotein-mediated phenomenon. The laboratory of Y. Becker reported inhibition of viral DNA polymerase in vitro (Fridlender et al., 1978) and this effect was taken to explain in vivo HSV inhibition as observed in their virus-cell system (Gordon et al., 1975; Shlomai et al., 1975). Gupta & Rapp (1976) interpreted their data on the allegedly specific failure of zinc-treated infected cells to synthetise certain viral proteins as an additional explanation for the inhibitory effect of ZnSO₄. Zinc uptake into cells is, however, subject to strict homeostatic control and it is for this reason that zinc, in contrast to cadmium, is well tolerated and even beneficial to the organism. Therefore it is unlikely that the inhibitory concentration for viral DNA polymerase could be reached in the nucleus of the infected cell.

In accordance with the work of Meshitsuka et al. (1987) we found that ZnSO₄ increasingly affects the culture cells when used in a concentration range from 50 to 200 μM. No inhibition of HSV replication could be observed below these limits. Only at 100 μM was virus synthesis considerably decreased, although viral as well as cellular DNA synthesis was sustained. At higher concentrations, all DNA synthesis in the infected cell as

The micrographs show clearly that zinc is deposited in considerable amounts onto virion structures, and that

step of negative staining with uranyl acetate was however omitted in two of the samples (insets in Fig. 9a).

Fig. 8. Kinetics of penetration and transport to the nucleus of native and ZnSO₄-inactivated HSV. Culture cells were infected at an m.o.i. of 10 with native or inactivated HSV-1 KOS radioactively labelled with [³H]thymidine. At the times indicated radioactivity of acid-precipitable material in the nuclei was determined as detailed in Methods. Native virus, △; inactivated virus, ●.
Zinc ions inactivate the virion of HSV

Well as cell morphology and presumably metabolic activity in general is severely affected.

Thus the early interpretation (Gordon et al., 1975; Shlomai et al., 1975) must be revised. Inhibition of HSV replication in culture cells is due to the cytotoxicity of the effective zinc concentration. The inhibitory effect of zinc ions on the formation of HSV DNA observed by Becker’s group might be explained as a secondary effect.
in the particular virus–cell system used, possibly due to non-specific inhibition of protein synthesis.

The concept that intracellular inhibition is the consequence of the cytotoxicity of ZnSO₄ rather than a specific interaction with viral synthetic processes is corroborated by results showing that zinc does not interfere with adsorption, penetration or egress of native virus, and that replication of different virus strains is inhibited to the same extent by the presence of ZnSO₄ in the culture medium. Free virus, however, is very effectively inactivated at the concentrations used in topical therapy (Holzmann et al., 1988). In control experiments, we clarified that this effect is indeed mediated by the zinc ions in the virus suspension.

Virion inactivation is independent of the counterion of the zinc salt and a possible pH shift due to the addition of the salt solution is without effect. Electron micrographs show that zinc does not simply precipitate or aggregate the HSV particles but the presence of the zinc ions leads to a considerable deposition of zinc ions in virion components. Since the majority of virus particles are non-infectious in a given suspension, information concerning the target component of zinc inactivation cannot be derived from the micrographs. Transport systems in viral membranes have not been described and are not to be expected; therefore interior structures should not be accessible to metal ions in intact virions. If only the virion surface interacts with zinc, membrane-bound surface glycoproteins are likely to be the target because membrane lipids are identical between virus strains, whereas glycoproteins are different. This could also explain differences in inactivation between viral strains. ZnSO₄ interacts with the virion only above the temperature range of transition of membranes from a rigid to a fluid state (Powers & Pershan, 1977; Chapman et al., 1974). This temperature effect is consistent with the idea that sensitive domains of the target glycoprotein have to be exposed by a conformational change. Indeed, ZnSO₄ inactivation of the herpes virion interferes with glycoprotein functions; virus penetration is severely affected whereas effects on adsorption are slight. These results lend themselves to the following interpretation. Membrane glycoproteins accumulate zinc non-specifically depending on zinc concentration and incubation time, possibly by binding to sulphydryl groups. The deposition of zinc interferes eventually with the function of HSV glycoprotein B or D, thus preventing virus penetration. This would be in line with conceptions of the mechanism of adsorption and penetration by membrane fusion. Adsorption can be considered to be a simple and insensitive process, mediated by several glycoproteins and independent of temperature and hence of active contributions from the cell metabolism. Penetration by membrane fusion in acidified vesicles, however, is thought to involve multiple interactions between viral glycoproteins and cell surface components (Fuller & Spear, 1985; Kühn et al., 1990). It appears to depend on energy transfer, physiological temperature and membrane fluidity. Thus, our finding that zinc inactivation affects penetration rather than adsorption, corroborates the interpretation that glycoproteins accumulate the metal until the zinc content becomes incompatible with the glycoprotein functions.

We conclude that the molecular mechanism of the therapeutic effect of ZnSO₄ in the treatment of herpetic lesions is not due to a cytovinvasive intracellular inhibition of virus replication, but to the drastic inactivation of free virus in skin tissues, intercellular vesicles and blisters.

References


(Received 14 March 1990; Accepted 28 August 1990)