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(54) Title: USE OF COPPER SILICATE FOR THE CONTROL OF HERPES INFECTIONS

(57) Abstract: The present invention relates to the use of copper silicate for treating and preventing Herpes virus (HV) infections and diseases and in particular diseases caused by HSV-1 or HSV-2 such as herpes and Varicella zoster (VZV) such as shingles. The present invention also relates to copper silicate compositions specifically adapted to treat HV infections.

Use of Copper Silicate for the Control of Herpes Infections

Field of the Invention

The present invention relates to the use of copper silicate for treating and preventing Herpes virus (HV) infections and diseases and in particular diseases caused by HSV-1 or HSV-2 such as herpes and Varicella zoster (VZV) such as shingles. The present invention also relates to copper silicate compositions specifically adapted to treat HV infections.

Background Art

Herpes simplex virus (HSV), Herpesviridae Simplexvirus, enters the host by direct contact, is spread to a target tissue only, spreads within the host via neuronal axonal flow, targets the dorsal root ganglia and after recovery of the host from an acute infection, remains latent in the targeted tissue.

HSV disrupts host cell molecular functions and host cellular structure and is manifested clinically as host cellular death, resulting in shallow, painful vesicular ectodermal lesions or by hemorrhagic encephalitic necrosis of the brain. Target tissues for HSV are the skin or mucous membranes usually derived from embryonic ectoderm: mouth, skin, vagina, conjunctiva, cornea, etc. The virus enters the host cell by direct mucosal contact or by direct contact of abraded skin. In the skin the virus replicates in epithelial cells and then enters local sensory neurons. The virus travels to the dorsal root ganglia via retrograde axonal flow where it establishes permanent residency. There it establishes latency a state in which the viral lytic genes are silenced and only the latency locus is transcriptionally active. Although latent most of the time, it reactivates intermittently, travels down the sensory nerve and causes vesicular eruptions at or near the site of initial invasion. Alternatively the virus may invade the CNS and cause encephalitis.

The rate of seropositivity to HSV varies widely from country to country: from relatively low in Japan where Herpes simplex Type 1 (HSV-1) seroprevalence for

men and women has decreased from 75.3 and 80.6% in 1973 to 54.4 and 59.6%, respectively in 1993 and where Herpes simplex Type 2 (HSV-2) seroprevalence has decreased from 10.2 and 9.9% in 1973 to 1.8 and 1.2%, respectively in 1993, to quite high in Africa where all adult study groups have a high HSV-1 seroprevalence of >80%. HSV infects more than 50% of the adult population, but some infections may be unrecognized. About half of these develop clinical manifestations of the disease. It's most significant manifestations are keratitis, genital lesions and labial vesicular lesions ("cold sores").

A common manifestation of HSV-1 infection is cold sores. However, HSV-1 also causes herpes keratitis (cornea). This disease is identified by a typically bizarre dendritic-patterned corneal ulcer that tends to be recurrent and very often leads to scarring with a reduction of vision, sometimes to the level of legal blindness. HSV-1 also causes herpes labialis, peri-orbital, peri-oral, peri-nasal skin eruptions and, in older patients, the virus has been associated with herpes zoster ("shingles") infection of the upper trunk.

HSV-2 causes the most prevalent sexually transmitted disease in the United States and visits to physicians for genital herpes simplex virus infection continue to increase. As many as 30 million Americans are infected with HSV-2. About half of these carriers are symptomatic. The clinical manifestations range from mild genital inflammation to severe, very painful, vesicular lesions and ulceration. Systemic involvement in the most severe cases may include hepatitis. Brain damage and death often are the result of HSV-2 acquired by a newborn infant as it passes through an infected birth canal. HSV-2 can also cause cold sores but this is relatively uncommon.

Aciclovir is used to treat HSV-1 and HSV-2 as well as other virus infections. The mode of action appears to be interference with viral DNA polymerase resulting in premature termination of the DNA chain and a reduction of viral replication. However, this agent is relatively expensive and there have been concerns of general cytotoxicity and the rapid, irreversible development of resistant viral strains. Furthermore, the topical version of Aciclovir is poorly absorbed dermally.

Varicella–zoster virus (VZV) is spread by the respiratory route and disseminates to lymph nodes and then via lymphocytes back to the skin, resulting in the rash of chickenpox. Like HSV, VZV infects the neurones of the dorsal root ganglia, where it causes lifelong latency. However, VZV reactivates much less often than HSV — in association with waning T-cell immunity — usually causing only one lifetime episode of herpes zoster (shingles). Nevertheless, VZV causes more severe damage to the nerve and dorsal root ganglia than HSV, leading to pain and often neural dysfunction. Prolonged pain may result from scarring of neural tissue.

Most people in developed countries are infected with VZV in childhood, with 90% seropositive by adulthood. Herpes zoster can develop at any age, but the highest incidence is after 60 years. Overall, it occurs in 20% of the population, with more than one recurrence in 4%.

The present invention seeks to overcome the above problems by providing a safe, relatively inexpensive and effective treatment for viral skin diseases such as those caused by HSV and VZV.

Summary of the Invention

The present invention provides for the use of copper silicate to control Herpes virus (HV).

The anti-HV activity of copper silicate renders it useful for treating HV infections. Thus, the present invention also provides a method of treating a HV infection in a subject, the method comprising the step of administering to said subject an effective amount of copper silicate.

The present invention further provides for the use of copper silicate for preparing a medicament for treating the clinical manifestations of HV infections such as cold sores (predominantly caused by HSV-1) or the sores associated with genital herpes (predominantly caused by HSV-2).

The present invention still further provides a method of treating or preventing HV infection in a subject, the method comprising the step of repeatedly topically administering an effective amount of copper silicate.

The copper silicate may be used as part of combination therapy. Thus, the present invention also provides a method of treating or preventing HV infection in a subject, the method comprising the step(s) of topically administering an effective amount of copper silicate and (ii) administering to said subject an effective amount of at least one other anti-viral agent or an effective amount of at least one anaesthetic.

The present invention also provides for the use of copper silicate for preparing a medicament for treating or preventing HV infection or a disease or disorder caused by HV.

The present invention further provides a composition adapted for topical administration to a site of HV infection comprising an anti-viral effective amount of copper silicate.

Brief Description of the Figures

Figure 1 is a graph comparing the anti-viral activity of the first copper silicate formulation and a copper sulfate solution;

Figure 2 is a graph depicting the anti-viral activity of different concentrations of the first copper silicate formulation over time;

Figure 3 is a graph comparing the anti-viral activity of the first copper silicate formulation and a copper sulfate formulation;

Figure 4 is a graph depicting the anti-viral activity of a second copper silicate formulation;

Figure 5 is a graph depicting the anti-viral activity of a copper silicate formulation according to one embodiment of the present invention against HSV1 in comparison to copper sulfate (Kill rate of 100% = no plaques observed);

Figure 6 is a graph depicting the anti-viral activity of a copper silicate formulation according to one embodiment of the present invention against HSV2 in comparison to copper sulfate (Kill rate of 100% = no plaques observed);

Figure 7 is a graph depicting the anti-viral activity of a copper silicate formulation according to one embodiment of the present invention against HSV2 ACR in comparison to copper sulfate (Kill rate of 100% = no plaques observed); and

Figure 8 is a graph depicting the cell toxicity of a copper silicate formulation according to one embodiment of the present invention in comparison to copper sulfate. Viability in % was assessed after 30 minute exposure to Cu (750-0.00125 ppm).

Detailed Description of the Invention

Use of copper silicate as an anti-viral

The present invention provides for the use of copper silicate to control herpes virus (HV).

For the purposes of the present invention, the phrase "controlling herpes virus" and similar phrases such as "control herpes virus" means one or more of the following: at least reducing the number of viable viruses; at least reducing viral replication; and at least reducing the clinical manifestations of HV infection such as reducing the number or size of lesions, reducing the pain associated with a clinical outbreak and/or reducing the healing time associated with the lesion.

For the purposes of the present invention HV includes HSV such as Herpes simplex virus 1 (HSV-1), Herpes simplex virus 2 (HSV-2), Varicella zoster virus

(VZV) and closely related viruses that cause similar infections/diseases in humans such as other members of the Alphaherpesvirinae subfamily.

The present invention also provides a method of treating a HV infection in a subject, the method comprising the step of administering to said subject an effective amount of copper silicate.

For the purposes of the present invention "HV infection" includes (i) clinical manifestations of HV infection in epithelial – derived tissues (mucous membrane or dermal origin) ; and (ii) sites where it is apparent that a clinical manifestation is likely to appear. Target tissues for HV are the skin or mucous membranes usually derived from embryonic ectoderm: mouth, skin, vagina, conjunctiva and cornea. Clinical manifestations include lesions e.g. cold sores and the lesions associated with genital herpes.

Particular disorders associated with HSV-1 infection that can be treated according to the present invention include: gingivostomatitis, genital and other cutaneous lesions, herpes keratitis (cornea), herpes labialis, peri-orbital, peri-oral, peri-nasal skin eruptions, keratoconjunctivitis, retinitis, oesophagitis, pneumonitis, hepatitis, meningitis, adult and neonatal encephalitis, myelitis, erythema multiforme. HSV-2 infection is associated with genital herpes with clinical manifestations ranging from mild genital inflammation to vesicular lesions and ulceration, genital and other cutaneous lesions, oesophagitis, pneumonitis, hepatitis, meningitis, adult and neonatal encephalitis, myelitis, erythema multiforme. Herpes varicella zoster virus is associated with chicken pox and shingles.

The copper silicate is preferably applied at a site of visible HV infection. For example, where a lesion exists or where it is apparent that a lesion will soon appear. In this regard, prior to the appearance of a cold sore, there may be localized reddening, tingling, itchiness and/or swelling that is indicative of HV infection. Thus, the present invention also provides for the use of copper silicate for preparing a medicament for treating the clinical manifestations of HV infections such as cold sores (predominantly caused by HSV-1) or the sores associated with genital herpes (predominantly caused by HSV-2).

Surprisingly, it has been found that the copper silicate has superior anti-viral activity against HSV-1 relative to other copper salts such as copper sulfate. It is also expected that the copper silicate products will have greater efficacy relative to other anti-virals including topicals for treating cold sores.

The copper silicate may be administered in a variety of ways but is preferably topically administered.

For the purposes of the present invention, the term "topical" and variants such as "topically" means application to a localized area of the body and/or to the surface of a body part and includes administration to the lips or mouth area, vagina (such as intra-vaginally) and surrounding areas, penis and surrounding areas and to mucous membranes.

Topical administration may be achieved in any one of a number of ways. Preferably, the copper silicate is administered by painting, wiping, rubbing, dabbing or spraying. Alternatively, topical administration may be achieved by applying the copper silicate using a means for dispensing an effective amount of copper silicate. Dispensing means can be varied and include slow release carriers and materials impregnated with copper silicate such as plasters, patches, bandages, cotton wool and gauze. Preferably, the dispensing means is adapted to deliver the copper silicate over a predetermined time and/or at a predetermined dose.

The amount of copper silicate administered in the method of the present invention will be sufficient to effectively treat the infection and thus will necessarily vary depending at least on the severity, type and location of the infection, the strength of the copper silicate and the manner in which individual patients respond to the treatment. Preferably, a copper silicate composition containing about 0.1 –10% wt copper is administered. The precise dose administered for a particular disorder may be determined by suitable qualified medical practitioner.

Similarly, the frequency with which, and the duration for which, the copper silicate is applied will be sufficient to effectively treat or prevent the infection and thus will

also vary depending at least on the severity and type of infection, the strength of the composition and the manner in which individual patients respond to the treatment. Preferably, the copper silicate is applied at least one, two, three or up to 5-10 times a day for at least 1 to 6 days or 1, 3, 6 or 12 weeks. Alternatively, it may be applied once every 2 – 14 days for as long as necessary to treat or prevent infection. The dosage regime for treating a particular disorder may be determined by suitable qualified medical practitioner.

Thus, the present invention also provides a method of treating or preventing HV infection in a subject, the method comprising the step of repeatedly topically administering an effective amount of copper silicate.

In some situations, it may be desirable to combine the copper silicate with other agents such as other anti-virals. Thus, the present invention also provides a method of treating or preventing HV infection in a subject, the method comprising the step(s) of topically administering an effective amount of copper silicate and (ii) administering to said subject an effective amount of at least one other anti-viral agent.

The other anti-viral agent may be varied and include systemic or topical agents or an agent selected from the list comprising: Aciclovir, Valaciclovir, Penciclovir, Famciclovir, Ganciclovir.

The copper silicate may also be combined with anaesthetics. Thus, the present invention also provides a method of treating or preventing HV infection in a subject, the method comprising the step(s) of topically administering an effective amount of copper silicate and (ii) administering to said subject an effective amount of at least one anaesthetic.

Preferably, the anaesthetic is a local anaesthetic. Even more preferably the local anaesthetic is of the ester type such as cocaine, procaine, tetracaine (amethocaine) and chloroprocaine, or the amide class such as lidocaine, prilocaine, mepivacaine, ropivacaine, levobupivacaine and bupivacaine.

The present invention also provides for the use of copper silicate for preparing a medicament for treating or preventing HV infection or a disease or disorder caused by HV.

Topical Formulations

The present invention also provides a composition adapted for topical administration to a site of HV infection comprising an anti-viral effective amount of copper silicate.

The form of the topical composition of the present invention may also be varied provided it retains its anti-viral properties. Preferably, the composition is a solution. However, the composition may also be in solid form provided it is properly formulated. In this regard, the composition could comprise copper silicate in the form of a micronized solid.

When the copper silicate is provided in the form of a solution, it preferably is provided as an aqueous acidified solution. Acidified solutions are particularly preferred because copper silicate is more soluble and stable at acidic pH. Particularly preferred pHs are 3-6, 4-6 and 5-6. In one example, the copper silicate is prepared according to the methods described and claimed in US patent 5,474,972.

The composition adapted for topical administration may be in the form of any one of the following: solution, lotion, suspension, emulsion, cream, gel, ointment, liniment and salve. Particularly preferred forms are ointments, creams or gels as these are more amenable to administration at the sites of HV infection.

Ointments generally are prepared using either (1) an oleaginous base, i.e., one consisting of fixed oils or hydrocarbons, such as white petroleum or mineral oil, or (2) an absorbent base, *i.e.*, one consisting of an anhydrous substance or substances that can absorb water, for example anhydrous lanolin. Customarily, following formation of the base, whether oleaginous or absorbent, the active ingredient is added to an amount affording the desired concentration.

Creams are oil/water emulsions. They consist of an oil phase (internal phase), comprising typically fixed oils, hydrocarbons and the like, waxes, petroleum, mineral oil and the like and an aqueous phase (continuous phase), comprising water and any water-soluble substances, such as added salts. The two phases are stabilised by use of an emulsifying agent, for example, a surface active agent, such as sodium lauryl sulfate; hydrophilic colloids, such as acacia colloidal clays, veegum and the like. For the purposes of the present invention, the copper silicate may be added to the water phase prior to formation of the emulsion, in an amount to achieve the desired concentration.

Gels comprise a base selected from an oleaginous base, water, or an emulsion-suspension base. To the base is added a gelling agent that forms a matrix in the base, increasing its viscosity. Examples of gelling agents are hydroxypropyl cellulose, acrylic acid polymers and the like. For the purposes of the present invention the copper silicate may be added to the formulation at the desired concentration at a point preceding addition of the gelling agent.

Excipients that can be incorporated into the gel, ointments and lotions of the present invention include: isopropyl myristate NF, trolamine NF, SD alcohol 40 (20%), white petrolatum USP, lanolin alcohols NF, mineral oil USP, polyvinyl alcohol gel, cetostearyl alcohol NF, lactic acid USP, sodium hydroxide USP, polysorbate 60 USNF, Cetyl alcohol USP, Mono- & Di- glycerides USNF, titanium dioxide calcium stearate, dextran, polyoxyl 40 stearate, methylparaben, propylene glycol, sodium lauryl sulfate, polyethylene glycol (PEG) base, synthetic beeswax (B wax), calcium acetate, purified water USP and similar products. These excipients serve a variety of functions as carriers, vehicles, diluents, binders, preservatives, buffers, pH adjusters, emulsifiers and other formulating aids.

Dosage forms of the invention for use in treating female genital manifestations of HSV infections, especially HSV-2, are prepared in dosage forms for vaginal insertion including vaginal suppositories, gels, creams and tablets. These preparations optionally may include one or more of the following suitable and pharmaceutically acceptable excipients, including but not limited to: isopropyl myristate NF, mineral oil USP, stearyl alcohol NF, benzoic acid USP, pegoxyl 7

stearate, methylparaben, propylparaben, propylene glycol, butylated hydroxyanisole, coconut or palm kernel oil triglycerides, polysorbate 60 or polysorbate 8, peglicol 5, PEG-100 stearate and sorbitan monostearate, calcium lactate, hydroxypropyl methylcellulose, polysaccharide carrageenan, corn starch, lactose, calcium lactate, silicon dioxide and purified water USP.

The topical formulations of the present invention may also be adapted to be delivered to the eye(s) of a subject. In this regard, the eye drop dosage form of the invention will optionally include one or more suitable and pharmaceutically acceptable inactive excipients, including but not limited to: preservatives from a group including benzalkonium chloride, methylparaben, edetate disodium, thimersol, chlorbutanol; buffers from a group including sodium citrate, potassium chloride, magnesium chloride, sodium acetate, citric acid, sodium lactate; vehicles from a group including polyvinyl alcohol, hydroxy methylcellulose, cetyl alcohol, carboxymethylcellulose, hydroxy-propylenemethyl cellulose; pH adjusters from a group including sulfuric acid, hydrochloric acid, sodium hydroxide, monosodium or disodium phosphate; purified water USP; poloxamer 407 or 188, polysorbate 80; polyoxyethylene polyoxypropylene compound; mineral oil USP.

The compositions of the present invention may be produced by dissolving or combining the copper silicate in an aqueous or non-aqueous carrier. In general, any liquid, cream, or gel, or similar substance that does not appreciably react with the copper silicate or any other active ingredient that may be introduced and which is non-irritating is suitable.

The composition of the present invention may further comprise an auxiliary agent such as any one or more of: preservatives, stabilizers, emulsifiers, wetting agents, fragrances, colouring agents, odour controllers and thickeners such as natural gums. However, it has been found that the copper silicate compositions of the present invention do not require the addition of preservatives to maintain an acceptable shelf-life.

The concentration of the copper in the composition may be varied depending at least on the severity and type of infection that the composition is to be used to

treat or prevent. However, preferably, the concentration of the copper is approximately 0.01 – 10%. More preferably, the copper concentration is to a final concentration of approximately 0.1 – 5 % or 0.2 – 3%. In one particular form, the concentration of copper is to a final concentration of about 0.22%.

General

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in the specification, individually or collectively and any and all combinations or any two or more of the steps or features.

The present invention is not to be limited in scope by the specific embodiments described herein, which are intended for the purpose of exemplification only. Functionally equivalent products, compositions and methods are clearly within the scope of the invention as described herein.

The entire disclosures of all publications (including patents, patent applications, journal articles, laboratory manuals, books, or other documents) cited herein are hereby incorporated by reference. No admission is made that any of the references constitute prior art or are part of the common general knowledge of those working in the field to which this invention relates.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

Other definitions for selected terms used herein may be found within the detailed description of the invention and apply throughout. Unless otherwise defined, all other scientific and technical terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which the invention belongs.

The present invention will now be described with reference to the following examples. The description of the examples is in no way to limit the generality of the preceding description.

Examples

General Materials/Methods

Copper silicate is made *in situ* by a combination of copper sulfate, and sodium silicate in an acidic solution. This solution can then be formulated further to solutions, creams, gels, etc as appropriate. As a result of the "in situ" production of the copper silicate, the contents of the copper silicate formulations listed here do not specifically contain "copper silicate" as one of the starting ingredients. Nonetheless, the copper silicate is present due to the reaction of the copper sulfate and the sodium silicate under the specified conditions.

Copper Silicate Solution (Formulation A)

Ingredient	%wt
Deionised Water	96.68
Copper sulfate pentahydrate	1.14
Acetic acid (90%)	0.93
Sodium silicate solution	1.20
Sodium Alkyl ether sulfate	0.05

Note: When used in the examples Formulation A was applied at pH 7 conditions

Copper Silicate Lotion (Formulation B)

Ingredient	%wt
Deionised Water	74.16
Copper sulfate pentahydrate	0.87
Acetic acid (90%)	0.72
Sodium silicate solution	0.92
Triethanolamine	1.10
Carbopol Ultrez 10	1.89
Glycerine	9.97
Alcohol	6.98
Sodium hydroxide solution 18%	3.39

Copper Sulfate / Acetate Solution (Formulation C)

Ingredient	%wt
Deionised Water	97.93
Copper sulfate pentahydrate	1.14
Acetic acid (90%)	0.93

Copper Silicate Cream (Formulation D)

This cream formulation is made in two steps, first by production of a concentrated copper silicate solution (I) that is then immediately on formulated into the final copper silicate cream.

Concentrated Copper Silicate Solution (I)

Material	Unit Formula (% by wt)
Sodium silicate solution	4.60
Purified Water	87.81
Copper sulfate pentahydrate	4.35
Acetic acid	3.24

Copper Silicate Cream (Formulation D)

Material	Quantity (% w/w)
Water Phase	
Concentrated Copper silicate solution	20.00
Purified water	41.20
Propylene glycol	9.00
Sodium hydroxide	q.s.
Oil Phase	
Polysorbate 60	7.40
Cetyl alcohol	7.40
Mono- & Di- Glycerides	7.40
Polyoxyl 40 stearate	7.10
Titanium dioxide	0.50

Example 1 – Anti-viral activity of copper silicate (Formulation A) compared to copper sulfate

Materials/Methods

HSV-1 was exposed, separately to Formulation A and a copper sulfate solution (each over a range of 175 – 1400 ppm Cu) for 30 min. The HSV/copper mixtures were diluted and layered onto the cell monolayer. After 1 hour the mixture was washed away and the cells incubated to allow plaques to form. Plaque numbers were reported as a percentage of the control.

Results

Copper silicate Formulation A began to kill HSV-1 at 600 ppm Cu and at 700 ppm the kill rate was >90% (Figure 1). By comparison, under these conditions, copper sulfate was still ineffective at 1400 ppm Cu (Figure 1).

Example 2. Anti-viral activity of copper silicate (Formulation A) assessed by exposure time.

Methods and Materials

The clinical isolate of HSV 1 was incubated with copper silicate (Formulation A) dilutions in the same way as example 1. After 30 min, 60 min and 120 min the mixture was washed away and the cells incubated to allow plaques to form. Plaque numbers were reported as a percentage of the control.

Results

Using copper silicate, when the exposure times were increased to 60 min, maximum kills of 99.9% were achieved with 700 ppm Cu and 99.999% with 800 ppm Cu (Figure 2).

Example 3 – Comparison of anti-viral activity of two copper formulations

Materials/Methods

A formulation similar to Formulation A but without the silicate component (Formulation C – effectively a mixture of copper sulfate and acetic acid in solution) was made and tested against Formulation A. The concentration used in the testing of both formulations was 700ppm Cu and the exposure times were 0-120 min in 30 min intervals.

Results

As the exposure time increased the percentage of virus particles killed by both formulations increased (Figure 3). The average percentage kill for Formulation A at 30 min is 80% compared to 50% for Formulation C. By 60 min copper silicate Formulation A achieved 99% kill, while Formulation C achieved 90% kill (Figure 3). At 120 min exposure Formulation A achieved >99.99% kill, which is a 2 log greater percentage kill than that seen for Formulation C (Figure 3).

Example 4- Anti-viral activity of copper silicate (Formulation B)

Materials/Methods

HSV-1 was exposed to Formulation B at various copper concentrations for 60 min in a similar protocol to that used for Example 1.

Results

A concentration of 1980 ppm Cu achieved a 99.9% kill (Figure 4). At 1100 ppm Cu and an exposure time of 60 min a 90% kill was achieved (Figure 4). This increased to 99.99% kill with 120 min exposure time.

Example 5 – in vitro comparison of copper silicate and copper sulfate

Materials/Methods

(i) Preparation of Vero Cells

Vero cells were grown to passage 25 and frozen with DMSO (dimethylsulfoxide). Reserve stocks were frozen at passage 24 to allow for extra stocks to be rapidly grown if needed. The cells were frozen and random samples were tested for viability. The stocks were shown to have good viability. The cells were used for the plaque assay at passage 27.

HSV1 and HSV2 as well as acyclovir resistant HSV2 (HSV2 ACR) were obtained from ECACC (European Collection of Cell Cultures) and grown up to produce 50 mL of each virus in a cell free medium. The final concentration of the virus in the initial stock aliquots were as follows:

1.6×10^7 pfu/mL for HSV1

6.1×10^5 pfu/mL for HSV2

2.7 x 10^5 pfu/mL for HSV2 ACR (stock 2: 6 x 10^5 pfu/mL for HSV2 ACR)

(ii) Copper solutions

1. Copper silicate solution (~ 0.28% w/w as Cu)

Ingredient	% by wt
Sodium silicate solution	1.15
Water	96.95
Copper sulfate pentahydrate	1.09
Acetic acid	0.81

2. Copper sulfate solution (~ 0.28% w/w as Cu)

Ingredient	% by wt
Copper sulfate pentahydrate	1.09
Water	98.91

Solutions of copper silicate and copper sulfate were supplied at a concentration of 2,800 ppm copper and a pH of approximately 4. The copper solutions required dilution to a maximum working concentration of 1,000 ppm and neutralisation of the acidity. Dilution and neutralisation were achieved by using Tris (hydroxymethyl) buffer (0.012g/L) and 0.425M NaOH. Neutralization produced distinct precipitates for copper silicate and copper sulfate, which were kept in suspension for the treatment of virus.

(iii) Plaque Assay (method)

The viral particles for HSV1, HSV2, and HSV2ACR were thawed and mixed at appropriate pfu/ml with the copper solutions at final copper concentrations of 750, 400, 200 100, 50, 25, and 0 ppm of copper to produce between 10 and 250 pfu per 24 well plate after dilution. The copper solutions and the viruses were incubated together for 30 minutes. The copper treated HSV was then diluted 1:500 to avoid toxicity on the Vero cells and plated on 24 well plates at concentrations between 10 to 250 pfu/well. The 24 well plates contained a monolayer of Vero cells in a supporting media. The resulting 500 fold dilution of the copper solutions took the concentration of copper down to 1.5, 0.8, 0.4, 0.2, 0.1, 0.05 and 0 ppm in 24 well plates.

Dimethyl cellulose (DMC) was added to the wells to help identify plaque formation. The plates were then incubated for 3 days to allow HSV plaque formation. Following incubation the fluid over the Vero cells was washed off and the Vero cells stained with crystal violet in 4% paraformaldehyde to inactivate the virus and identify defects in the Vero cell monolayer caused by focal HSV replication (virus plaque). Plaques were counted with 5 fold digital magnification of each well after scanning of stained 24 well plates. There were 7 solutions for each viral strain and each solution was duplicated 4 times on 24 well plates.

Each plaque assay was repeated at least 8 times.

(iv) Cell toxicity (Method)

The cytotoxic effect of copper compounds was tested on Vero cells prior to plaque reduction assays using the CellTiter-Blue™ (Promega) Cell viability assay, followed by colorimetric analysis in 96 well plates.

Toxicity of high concentration Cu solutions was tested in 2 independent experiments. Cu treated virus solutions were tested for cytotoxicity in 96 well plates in parallel to each plaque assay.

Results

(i) Plaque Assays

Antiviral activity against HSV1 of soluble copper silicate became evident at 25 ppm Cu with a kill rate of 48% and at 400 ppm the kill rate was 100 % (Figure 5) following an exposure time of 30 minutes. By comparison, under these conditions, the kill rate of copper sulfate was only 9% at 25 ppm Cu and only achieved a kill rate of 100% at 750 ppm Cu.

Antiviral activity against HSV2 of soluble copper silicate became evident at 25 ppm Cu with a kill rate of 57% and at 400 ppm the kill rate was 100 % (Figure 6) following an exposure time of 30 minutes. By comparison, under these

conditions, the kill rate of copper sulfate was only 35% at 25 ppm Cu and copper sulfate did NOT achieve a kill rate of 100% at the Cu concentrations tested.

Antiviral activity against HSV2 ACR of soluble copper silicate became evident at 25 ppm Cu with a high kill rate of 84% and at 400 ppm the kill rate was 100 % (Figure 7) following an exposure time of 30 minutes. However, by comparison, under these conditions, the kill rate of copper sulphate was almost identical with 82% at 25 ppm Cu. Copper sulphate did achieve a kill rate of 100% at 750 ppm Cu.

Exposure times up to 3 days lead to 100% HSV virus kill rates at Cu concentrations of 25 ppm and lower (results not shown).

(ii) Cell Toxicity

Toxicity of soluble copper silicate became evident (less than 100% cell vialbility) at 0.0125 ppm Cu (Figure 8) following an exposure time of 30 minutes. Under these conditions, copper sulphate was toxic at 0.00125 ppm Cu and more toxic than copper silicate throughout.

Cu toxicity interfering with plaque assays (less than 50% cell viability) could be observed at Cu concentrations down to 1.5 ppm (750 ppm 1:500 diluted) after 3 days of exposure (= duration of the plaque assay). 1:500 diluted Cu solutions prior to plaque assay showed minimal cytotoxicity allowing for read-out of plaque assays at concentrations of 2 to 1.5 ppm (1000 ppm to 750 ppm Cu-virus solutions).

The Claims defining the Invention are:

1. Use of copper silicate to control Herpes virus.

2. Use according to claim 1 wherein the Herpes virus is HSV-1 or HSV-2.

3. Use according to claim 1 wherein the Herpes virus is Varicella zoster virus.

4. A method of treating a Herpes virus infection in a subject, the method comprising the step of administering to said subject an effective amount of copper silicate.

5. A method according to claim 4 wherein the Herpes virus infection is a HSV-1 or HSV-2 infection.

6. A method according to claim 4 wherein the Herpes virus infection is A Varicella zoster infection.

7. A method according to claim 5 wherein the HSV infection is in epithelial – derived tissues (mucous membrane or dermal origin)

8. A method according to claim 5 wherein the HSV infection is in the skin or mucous membranes.

9. A method according to claim 8 wherein the infection is at a site selected from the group of sites consisting of mouth, skin, vagina, conjunctiva and cornea.

10. A method of treating a disorder associated with Herpes virus infection in a subject comprising administering to said subject an effective amount of copper silicate.

11. A method according to claim 10 wherein the disorder is selected from the group of disorders consisting of: gingivostomatitis, genital and other cutaneous lesions, herpes keratitis (cornea), herpes labialis, peri-orbital, peri-oral, peri-

nasal skin eruptions, keratoconjunctivitis, retinitis, oesophagitis, pneumonitis, hepatitis, meningitis, adult and neonatal encephalitis, myelitis, erythema multiforme, genital herpes with clinical manifestations ranging from mild genital inflammation to vesicular lesions and ulceration, chicken pox and shingles.

12. Use of copper silicate for preparing a medicament for treating the clinical manifestations of Herpes virus infections such as cold sores or the sores associated with genital herpes.

13. A method according to any one of claims 4-11 wherein the copper silicate is administered topically.

14. A method according to any one of claims 4-11 or 13 or a use according to any one of claims 1-3 or 12 wherein about 0.1 –10% wt copper is administered or used.

15. A method according to any one of claims 4-11 or 13-14 or a use according to any one of claims 1-3 or 12 wherein the copper silicate is administered or used at least once a day for at least 1 to 6 days.

16. A method of treating or preventing Herpes virus infection in a subject, the method comprising the step of repeatedly topically administering an effective amount of copper silicate.

17. A method of treating or preventing Herpes virus infection in a subject, the method comprising the step(s) of topically administering an effective amount of copper silicate and (ii) administering to said subject an effective amount of at least one other anti-viral agent.

18. A method according to claim 16 or 17 wherein the Herpes virus infection is caused by HSV-1, HSV-2 and/or Varicella virus.

19. A method according to claim 17 or 18 wherein the other anti-viral agent is selected from the list consisting: Aciclovir, Valaciclovir, Penciclovir, Famciclovir and Ganciclovir.

20. A method of treating or preventing Herpes virus infection in a subject, the method comprising the step(s) of topically administering an effective amount of copper silicate and (ii) administering to said subject an effective amount of at least one anaesthetic.

21. A method according to claim 20 wherein the Herpes virus infection is caused by HSV-1, HSV-2 and/or Varicella virus.

22. A method according to claim 20 or 21 wherein the anaesthetic is selected from the group consisting of: cocaine, procaine, tetracaine (amethocaine), chloroprocaine, lidocaine, prilocaine, mepivacaine, ropivacaine, levobupivacaine and bupivacaine.

23. Use of copper silicate for preparing a medicament for treating or preventing Herpes virus infection.

24. Use of copper silicate for preparing a medicament for treating or preventing a disease or disorder caused by Herpes virus.

25. Use according to claim 23 or 24 wherein the Herpes virus infection is caused by HSV-1, HSV-2 and/or Varicella virus.

26. A composition adapted for topical administration to a site of Herpes virus infection comprising an anti-viral effective amount of copper silicate.

27. A composition according to claim 26 wherein the concentration of the copper in the composition is approximately 0.01 – 10%.

Figure 1

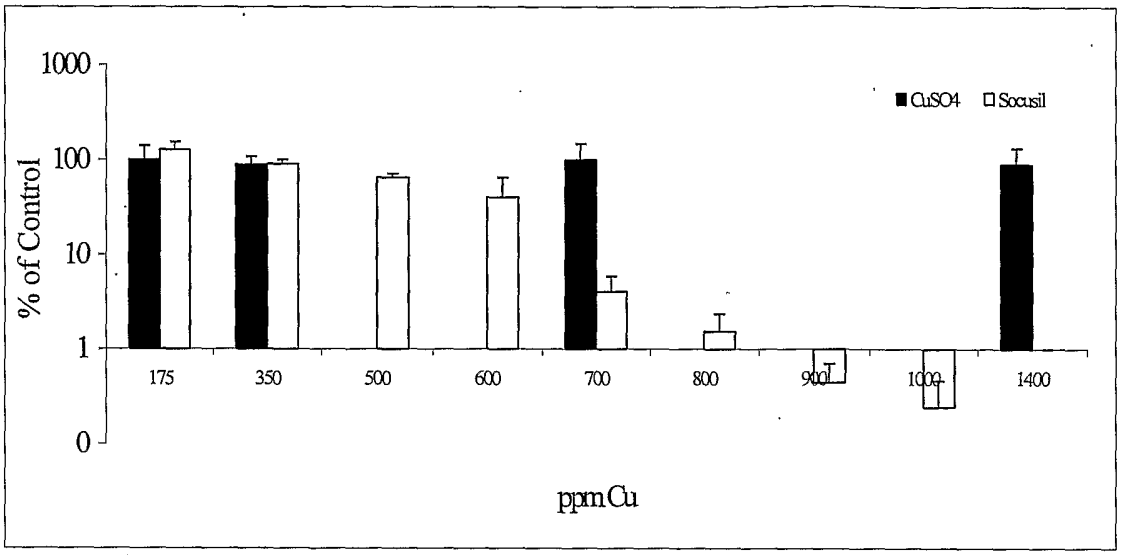


Figure 2

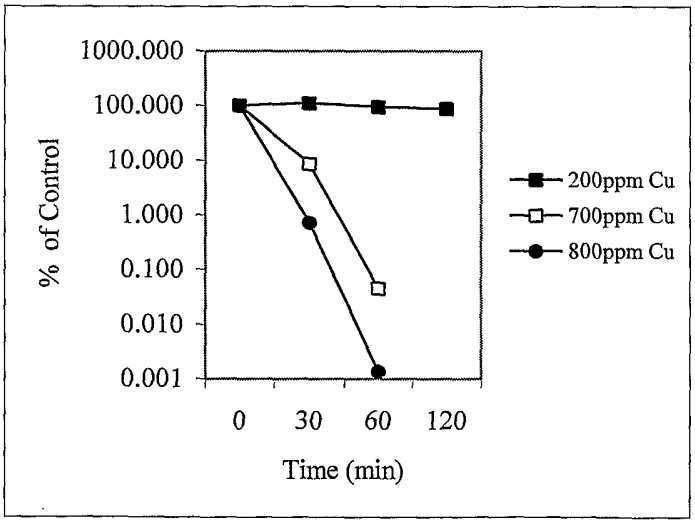


Figure 3

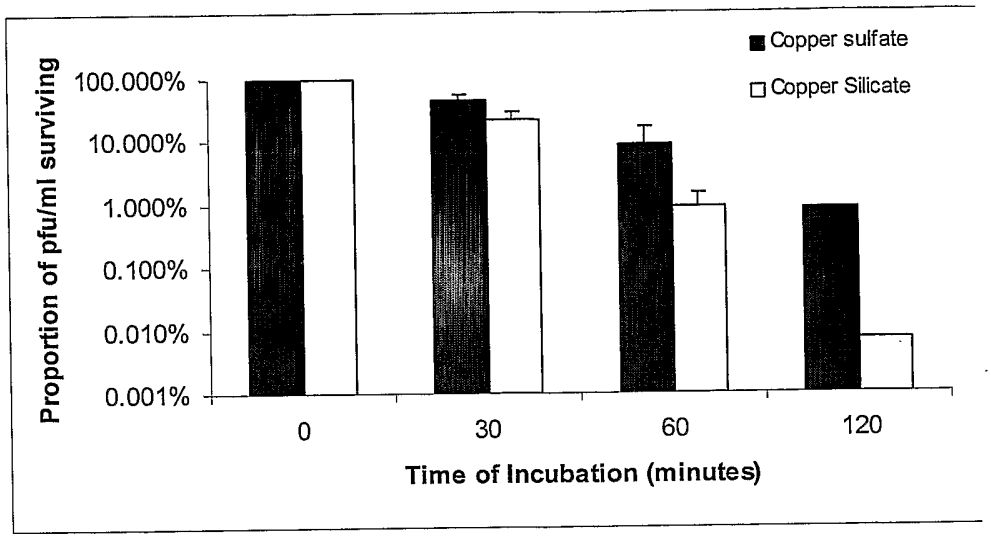


Figure 4

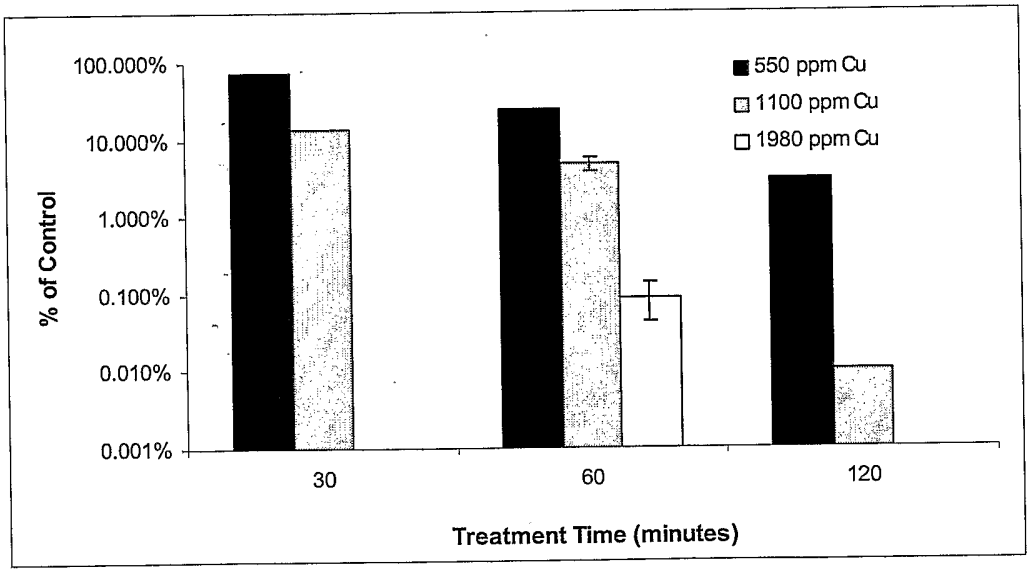


Figure 5

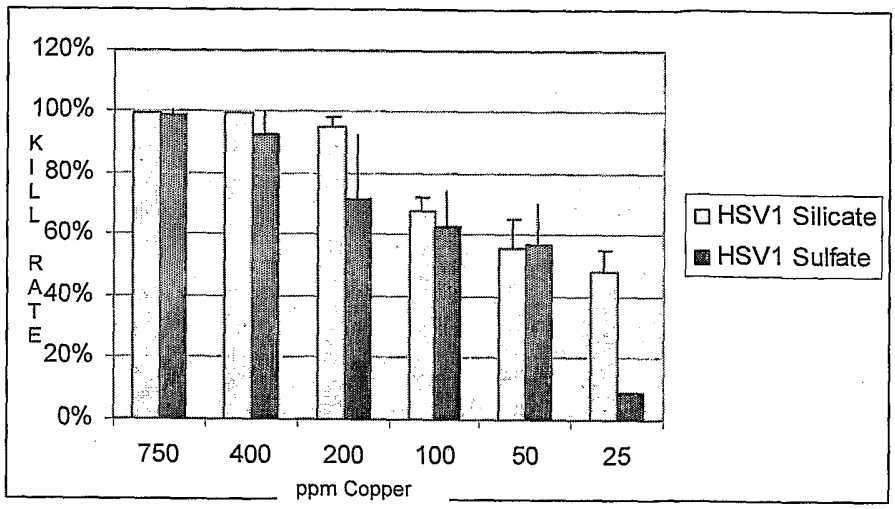


Figure 6

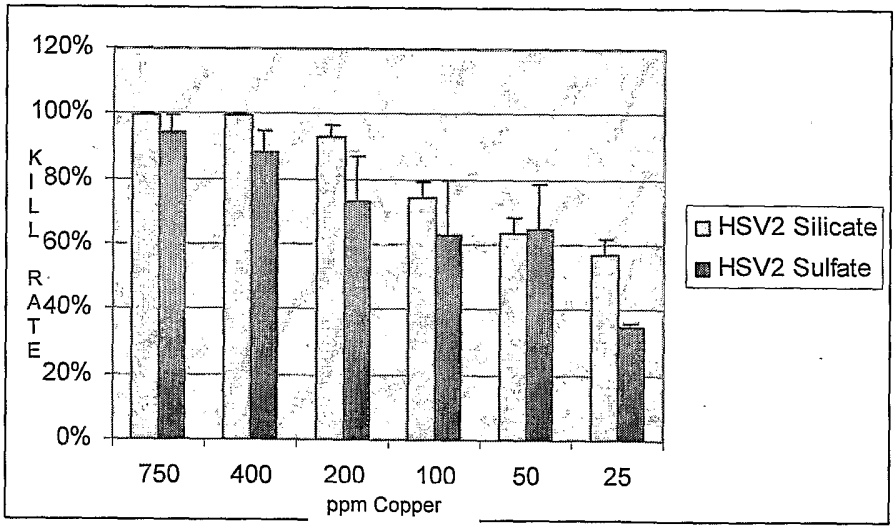


Figure 7

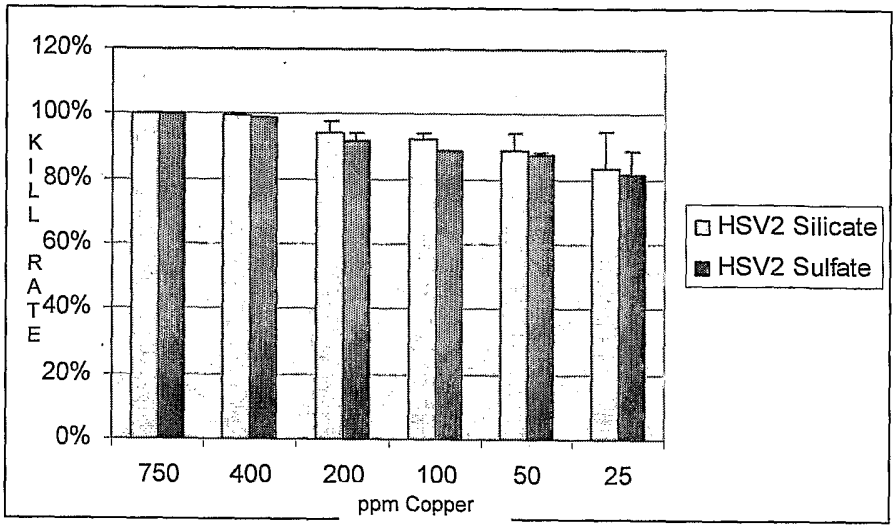
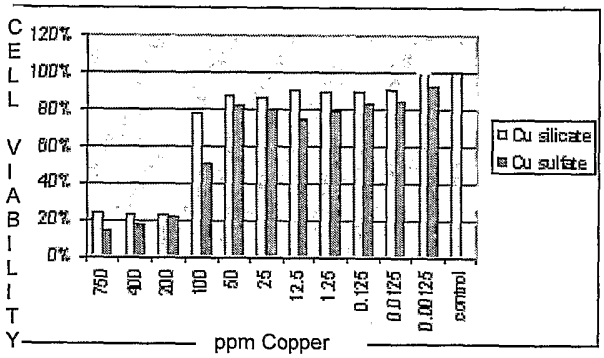


Figure 8



INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2006/000363

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.

A61K 33/34 (2006.01) *A61P 31/22* (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
DWPI, CAPlus, Medline: copper silicate, herpes, varicella, cold sore, VZV, HSV

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2003/088983 A (CONVE LTD.) 30 October 2003 See whole document	26 to 27
X	WO 1999/027942 A (SHEEN BIOTECHNOLOGY PTY. LTD.) 10 June 1999 See whole document	26 to 27
X	WO 1993/007754 A (SHEEN, R.J. et al.) 29 April 1993 See whole document	26 to 27

☐ Further documents are listed in the continuation of Box C ☒ See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
31 March 2006	7 APR 2006
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized officer **Michael Grieve** Telephone No : (02) 6283 2267

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2006/000363

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
WO	03088983	AU	2003227095	BR	0309266	CA	2482439
		CN	1649605	EP	1494688	MX	PA04010131
		NZ	535903				
WO	9927942	AU	16474/99				
WO	9307754	AU	27621/92	CA	2121327	EP	0609285
		SG	47493	US	5474972		

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

END OF ANNEX