Novel Nanoviricides® Highly Effective Against Varicella Zoster Virus in Cell Culture

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Abstract

Varicella Zoster virus (VZV) primary infection causes chickenpox, followed by latency in ganglia and neurons, and can reactivate decades later causing herpes zoster (shingles), usually upon immunosuppression resulting from age, stress, or other factors. Classical shingles presents as a painful unilateral dermatomal vesicular rash as virus spreads to the skin through peripheral nerves. In severe cases, VZV can reactivate in or around the trunk which can cause facial disfiguration or blindness. There are about 1 million cases annually and the lifetime risk of developing shingles is at least 30%. While there is a shingles vaccine, it is not effective post-breakout, is only ~50% effective in preventing disease, and cannot be given to immunosuppressed people. Topical treatment of shingles remains an unmet medical need, and would enable high concentration of active drug locally for rapid treatment with minimal systemic effects. NanoViricides, Inc. is developing broad-spectrum drugs against herpesviruses for both topical and systemic use.

Nanoviricides, Inc. is developing different broad-spectrum drugs against Varicella Zoster virus (VZV) to address these issues. Our novel nanoviricide class of drug candidates are designed to specifically attack enveloped virus particles by using small chemical ligands that mimic the surface of the viral membrane, thereby disrupting the virus envelope and inhibiting viral entry into the host cell. This approach allows for the development of novel antivirals that are effective against multiple strains of the virus and can be used topically, systemically, and in combination with other antiviral drugs. In this study, we evaluated the efficacy of our nanoviricides against VZV using in vitro and in vivo models.

Introduction

There are eight herpesviruses known to infect humans. Varicella Zoster virus (VZV) is a member of Herpesviridae and is also known as human herpesvirus 3 (HHV-3). Primary infection with VZV causes chickenpox in children, followed by latency in nerves, including the cranial ganglia, dorsal root ganglia, and autonomic ganglia, and can reactivate decades later to cause herpes zoster (shingles) in adults usually upon immune compromise resulting from age, stress, or other factors. Classical shingles presents as a painful unilateral dermatomal rash caused by reactivation of the virus in the peripheral nervous system. The pain is often severe and may extend to the skin. There is currently no effective treatment for shingles, although antiviral drugs such as acyclovir can reduce the duration and severity of symptoms. However, these drugs do not cure the disease and can have significant side effects.

Methods

CELL-BASED ELISAs: We have successfully developed a cell-based ELISA in 96-well black, clear-bottom plates which allows us to perform high-throughput screening of compounds to compare other methods. With appropriate antibody selection, we have been able to apply this ELISA technique to quantification of different viruses. In the VZV assay presented here, VZV is pre-incubated with compounds and then added to ARPE-19 cells and incubated for 6 days (see Figure 1 and 2). This gives the virus time to replicate and express viral proteins. Cells are then fixed in formalin, so viral protein expression can be measured and compared to uninfected controls. We can compare infected cells, infected cells with no treatment, and cells treated with our compounds or positive controls such as Acyclovir Sodium (ACV Na+). We can then compare relative infection levels between all groups in terms of quantification of reduction in protein expression. A reduction in protein expression compared to untreated, infected controls suggests a decrease in virus quantity, and serves as a quantitatively comparable estimate of virus production/growth/spread.

Results

Figure 3: Initial screening of multiple compounds. In order to narrow down our list of in-house compounds to determine which are effective against VZV, we used our cell-based ELISA to screen many compounds. ARPE-19 cells were seeded in 96-well plates 24 hours prior to infection. VZV was incubated with 4 different concentrations of each compound for 1 hour prior to infection of the cells. After 6 days, plates were fixed in formalin. Cell-based ELISAs were performed and percent infection relative to untreated infected controls was determined by measuring absorbance.

Figure 4: Compounds NV-118 and NV-121 are non-toxic to cells in vitro. In order to determine if our compounds, compounds NV-118 and NV-121, were toxic to the cells themselves, we performed a cell-viability assay. ARPE-19 cells were seeded in 96-well plates 24 hours prior to addition of 4 different concentrations of compounds. After 6 days (same day as the plates were fixed for ELISA measurement), cell viability was determined by Promega’s CellTitre-Glo Luminiscence Cell Viability Assay. These are the combined results of 2 separate experiments (n=6). Selected compounds are shown.

Figure 5: Compounds NV-118 and NV-121 are extremely effective inhibiting VZV in vitro. In contrast, NV-93 was less effective, ARPE-19 cells were seeded in 96-well plates 24 hours prior to infection. VZV was incubated with 4 different concentrations of compounds, inhibitors, and vehicles for 1 hour prior to infection of the cells. After 6 days, plates were fixed in formalin. Cell-based ELISAs were performed and percent infection relative to untreated infected controls was determined by measuring absorbance. Combined results of 2 separate experiments (n=6) shown.

Conclusions

• We present here two nanoviricide® active drug candidates, NV-118 and NV-121, that are highly effective against VZV in vitro, and one, NV-93, that was comparatively ineffective. The three candidates differ only in the chemical structures of their ligands, demonstrating ligand-directed virus specificity.

• All three compounds were found to be non-cytotoxic in ARPE-19 cells as well as every other cell line tested.

• These two active candidates were found to inhibit VZV up to 5 times better than acyclovir-sodium treatment (the current standard of care) in vitro, and completely inhibited VZV protein production/infection at the highest dose.

Future Directions

NanoViricides, Inc. is now advancing these two drug candidates further into ex vivo dermal studies towards IND filing. NanoViricides, Inc. is now advancing these two drug candidates further into kg-scale, clinical studies.

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