

Introduction
Methods

Results
Conclusion

Introduction

As the commonly known causative agent of cold sores, Herpes simplex virus type 1 (HSV-1) can also lead to devastating conditions such as encephalitis and blindness. HSV-1 currently affects over two-thirds of people worldwide.¹ There are two types of HSV; type 1 which generally causes oral herpes and type 2, which leads to genital herpes. HSV-1 initially infects epithelial cells, including keratinocytes. Following primary infections, HSV can travel through sensory neurons to neural ganglia where the virus evades the immune system and remains throughout the lifespan of an infected individual.

HSV has an intricate life cycle involving many different host and viral proteins, and thus providing several possible targets for potential antiviral therapies. Current therapies for HSV, such as Acyclovir, target the virus protein DNA polymerase. Long term use of these therapies can lead to resistance, increasing the need for anti-viral therapies that target different parts of HSV's life cycle.² HSV has been shown to increase host telomerase activity.³ MST-312 is a telomerase inhibitor, which has been previously shown to significantly reduce HSV replication in immortalized cell lines.⁴ However, MST-312 has yet to be tested in HSV infected primary cells.

The purpose of this study was to assess MST-312's ability to inhibit HSV infection in primary human cells, including keratinocytes. Viral assays were performed to determine the ability of MST-312 to prevent HSV progeny production and cell lysates were immunoblotted for viral proteins to determine reduction in viral protein synthesis. Trypan blue staining was also performed to assess the level of cytotoxicity of MST-312 treatment.

Methods

Cells and Virus

Human neonatal keratinocytes (nHEK) were maintained in serum free keratinocyte media. Vero cells, which are a monkey kidney epithelial cell line, were used as a control and maintained in DMEM supplemented with 5% FBS. The strain of HSV-1 used was KOS 1.1.

Chemicals

A 10mM MST-312 (EMD Millipore) stock was prepared in DMSO and aliquots frozen at -20°C until ready to use. Cells were treated with 20 µM MST-312 or an equivalent volume of DMSO, as a control.

Immunoblotting

Cells were infected with HSV-1 at an MOI of 5 in the presence of 20 µM MST-312 or equivalent volume of DMSO. At 18 hpi, cells were harvested by scraping the cells into the media. Cell pellets were prepared through centrifugation. Cell pellets were lysed with radioimmunoprecipitation (RIPA) buffer and lysates were quantified using a Bradford Assay. 15 µg of protein lysate from each sample was separated using a 12% SDS-polyacrylamide gel electrophoresis (PAGE). The gel was transferred to a nitrocellulose membrane and immunoblotted for viral proteins (gC, ICP27 and VP22) and a cellular protein (actin).

Plaque Assays

Cells were infected with HSV-1 at an MOI of 5 in the presence of 20 µM MST-312 or equivalent volume of DMSO. At 18 hpi, the infected monolayers were frozen at -80 ° C. Following thawing, the infected cells were scraped into the media. Sterile milk was then added to each viral sample, creating a concentration of 50%. Virus was released through sonication. 10-fold serial dilutions (10⁻¹ to 10⁻⁷) of the virus samples were prepared and added to confluent Vero cells in 6-well plates. After two hours of incubation at 37°C, the media containing the virus was replaced with fresh media containing pooled human immunoglobulin. Cell plates were then incubated at 37 degrees for three days. Following incubation, the cell plates were rinsed, fixed with methanol, and stained with Giemsa. Plaques were counted through visual inspection and microscopy.

Trypan Blue Cytotoxicity Testing

MST-312 or DMSO was added to the media of nHEK cells, and cells were incubated for 18 hours at 37°C. Following incubation, the cells were trypsinized and stained with trypan blue dye. Trypan blue is selectively taken up by dead cells, thus allowing cells to be counted under light microscopy using a hemacytometer. The percentage of dead cells was calculated by dividing the number of blue cells by the total number of cells.

Statistical Analysis

Cytotoxicity and plaque assay experiments were performed with triplicate samples. A two-tailed, unpaired student's t-test was utilized to determine statistical significance. A p-value of 0.05 or below was considered significant.

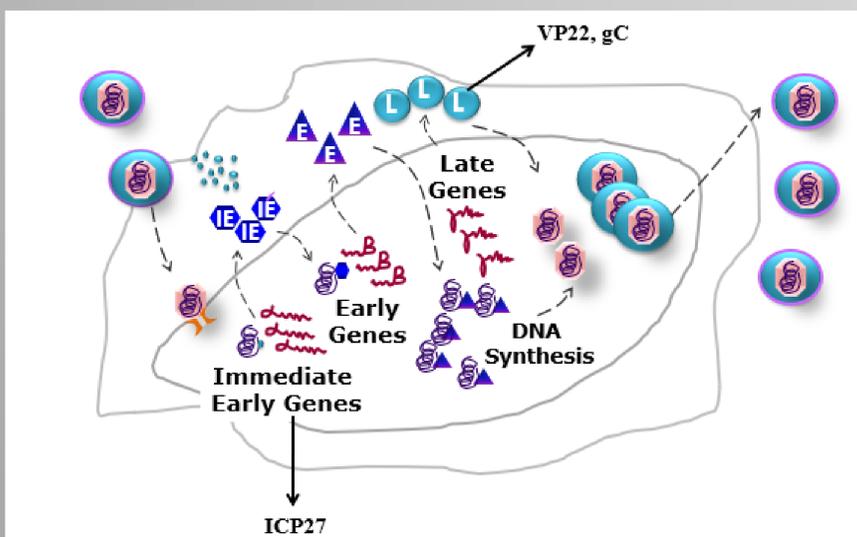


Figure 1: HSV life cycle: The HSV life cycle starts with fusion of the HSV envelope proteins with the host cell membrane. HSV then inserts its capsid into the cell. The capsid travels to the nucleus and releases its genome. The genome utilizes host cell factors to trigger immediate early gene expression. First, immediate early genes are transcribed and translated, which then leads to early gene expression, followed by late gene expression.

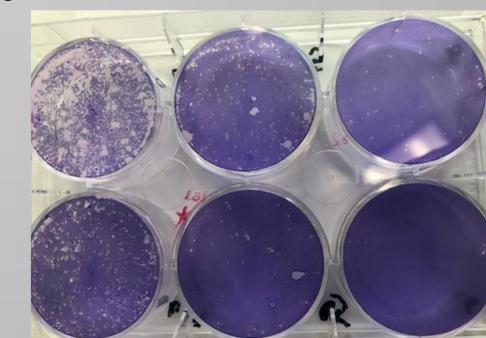
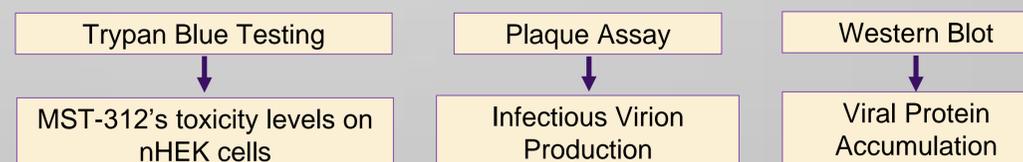


Figure 2: Plaque Assay. Cell plate stained with Giemsa, which allows for quantification of virion production in plaque assay experiment.

MST-312 Suppresses Herpes Simplex Virus Replication in Primary Human Cells

Alyssa J. Averhoff, Prajakta Pradhan, Marie L. Nguyen, PhD

Des Moines University

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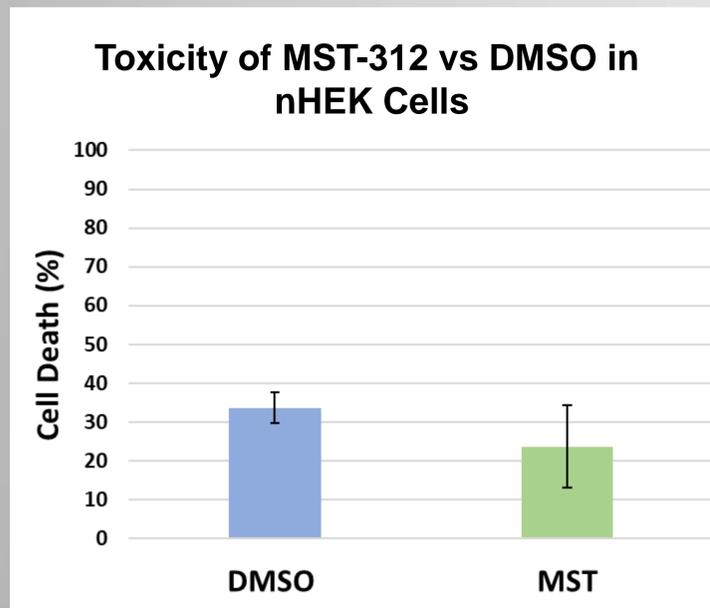


Figure 3: MST-312 induces a similar level of cytotoxicity to DMSO controls in nHEK cells. 20 μ M of the inhibitor, MST-312, or the equivalent volume of DMSO was added to nHEK cells. 18 hpi, cells were trypsinized and stained with trypan blue. Dead cells and live cells were counted under light microscopy. The bars represent the average percent cell death of three samples and the error bars represent standard deviation. No statistically significant difference in cell death is seen with MST-312 compared to control DMSO in nHEK cells.

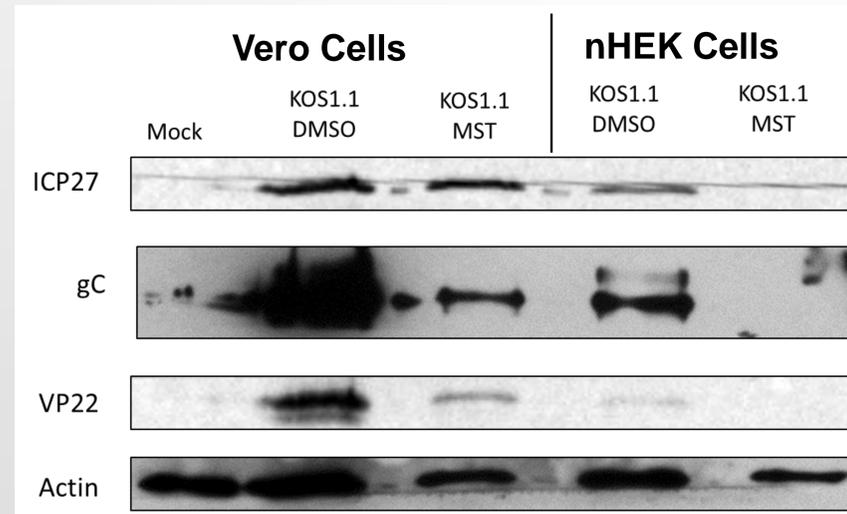


Figure 4: MST-312 suppresses viral protein accumulation in Vero and nHEK cells. Vero cells and keratinocytes were infected with HSV KOS1.1 at an MOI of 5 in the presence of 20 μ M MST-312 and equivalent volume DMSO. A mock infection control was done in Vero cells. Cells were harvested at 18 hpi and lysed for protein analysis. 15 μ g of each protein lysate was separated on a SDS-PAGE gel which was then immunoblotted for viral proteins gC, ICP27, and VP22. Membranes were also immunoblotted for the cellular protein, actin, as a control. Results indicate reduction in late viral protein accumulation (VP22 and gC) with MST-312 treatment compared to control DMSO in Vero cells and keratinocytes. Immediate early protein ICP27 was reduced in the presence of MST-312 in nHEK cells, however it was not reduced in MST-312 treated Vero cells.

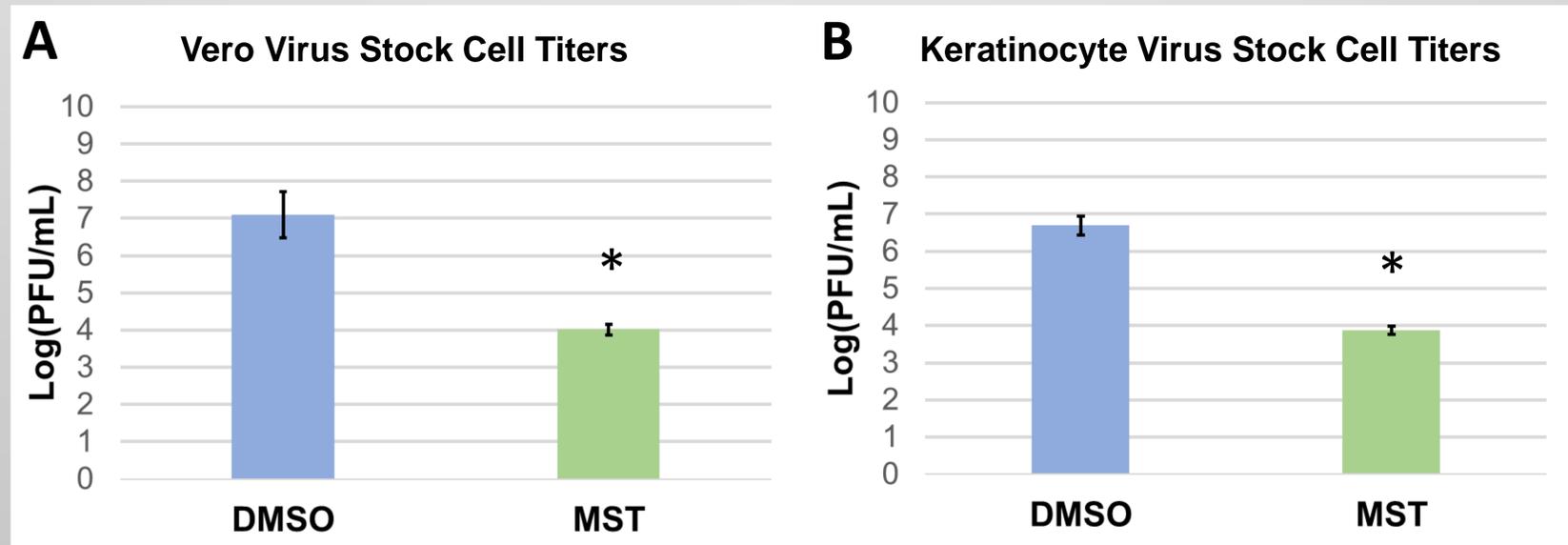


Figure 5: MST-312 inhibits virion production in keratinocytes. Vero cells (A) and Keratinocytes (B) were infected with HSV-1 in the presence of 20 μ M MST-312 or equivalent volume of DMSO. At 18 hpi, virus was harvested and quantified via plaque assay on Vero cells. For both graphs, the bars represent the averages and the error bars indicate the standard deviation. Results indicate a significant decrease in virion production with both Vero cell and nHEK cell virus stock. * $p < 0.05$ based on Student's T-test

Conclusions

- Cytotoxicity of MST-312 treated nHEK cells was similar to DMSO control.
- MST-312 inhibits HSV-1 protein accumulation in nHEK cells.
- MST-312 reduces HSV-1 virion production in nHEK cells.

References

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