MRCHS122

Control of Herpes Simplex viral infection by manipulation of viral capsid and tegument protein expression

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Introduction

Herpes Simplex viruses (HSV- & HSV-2) affects 2/3rd of world-wide population. HSV raises the risk of life-threatening diseases like HIV in immunocompromised patients.

There is no vaccine available to prevent HSV infections yet.

The present study will contribute to HSV research by selecting alternative target specific therapies focusing on different targets belonging to various viral functional communities.

Herpes viral encapsidation proteins is gaining interest in HSV research.

This study proposes to apply with different approach on inhibiting viral encapsidation gene that encodes capsid transport tegument protein (CTTP) to achieve greater success. This gene is highly conserved in HSV-1 and HSV-2 making it feasible to design a single therapy for two viruses.

RNAi therapy is the main modality anticipated to reveal predicted synergistic effect, that may cap the overuse of standard antivirals such as Acyclovir (ACV) and its related side effects.

Aim

The present study has utilized RNAi therapy exclusively to focus on two aspects –

1) providing a unified therapy to inhibit both HSV-1 and HSV-2 simultaneously.
2) secondly evaluation of combination therapy of siRNA with standard drug.

Objectives

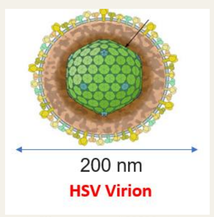
1. Homology analysis of viral target, designing of siRNA against viral encapsidation gene and delineating the protein-protein interactions of CTTP.
2. Evaluation of anti-HSV activity of developed siRNA by cell culture models.

i. Comparative screening of designed siRNAs (S1 and S2) against HSV-1 & HSV-2 in two different cell culture models Vero and HaCaT.

ii. Combination testing of standard ACV and selected siRNA against HSV-1 & HSV-2 in Vero and HaCaT

iii. Effect of combination study in Plaque reduction assay.

Hypothesis



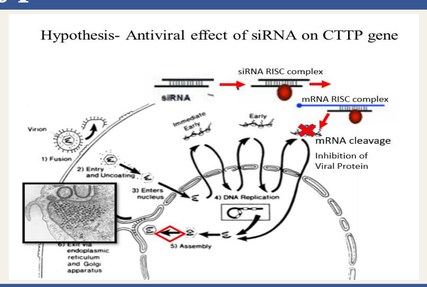


Figure 1: Illustration of HSV virion and target viral protein mechanism with study hypothesis

Methodology

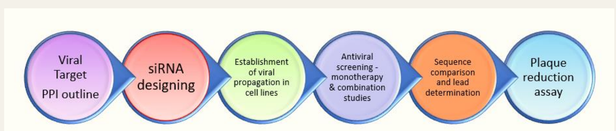


Figure 2: Brief outline of methodology

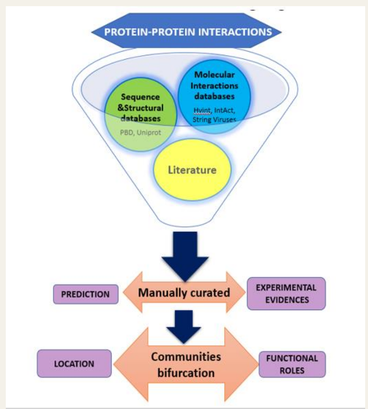


Figure 3: Scheme for outlining the PPI of viral CTTP

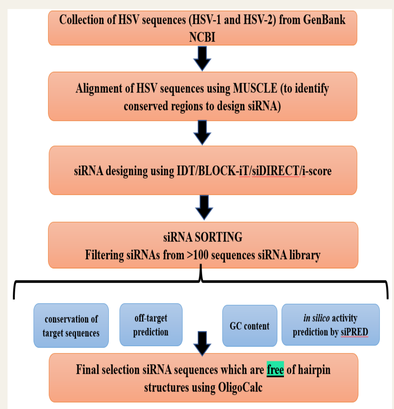


Figure 4: Designing of siRNA targeting HSV CTTP

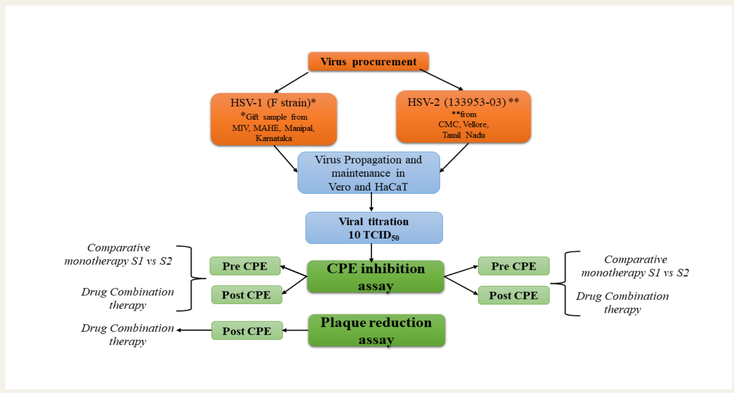


Figure 5: In Vitro methodology flowchart

Highlights of the study

- ✓ Two siRNAs SI and S2 were preliminarily screened for viability in both Vero and HaCaT in a dose range of 1-100 nM. The higher doses were well tolerated by both cell lines (data not represented).
- ✓ Viral titer of 10TCID$_{50}$ HSV-1 (10^{-6}) and HSV-2 (10^{-4}) were used for this study.
- ✓ IC$_{50}$ of naked siRNA was determined at 30 nM and 34 nM for HSV-1 and HSV-2 respectively. Comparative antiviral screening of siRNAs against both HSV-1 and HSV-2 was performed in the dose range of 10-50 nM.
- ✓ Based on available literature and supported by preliminary screening, doses of Acyclovir (ACV) were set at IC$_{50}$ (2.5-5 µg/mL) and at IC$_{10}$ (1-2µg/mL) in monotherapy and combination studies, respectively.
- ✓ A single dose of standard drug ACV at IC$_{10}$ combined with a single dose siRNA at IC$_{10}$ (i.e., 5 nM) were used in a ratio of (1:2) in combination studies.
- ✓ Antiviral activity was evaluated using SRB assay.

Results

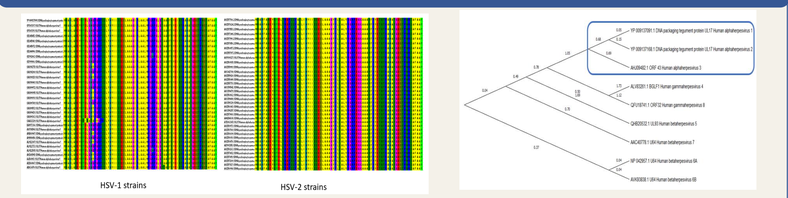


Figure 6: Homology analysis of CTTP in HSV strains

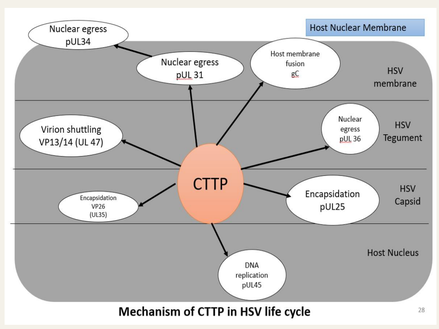
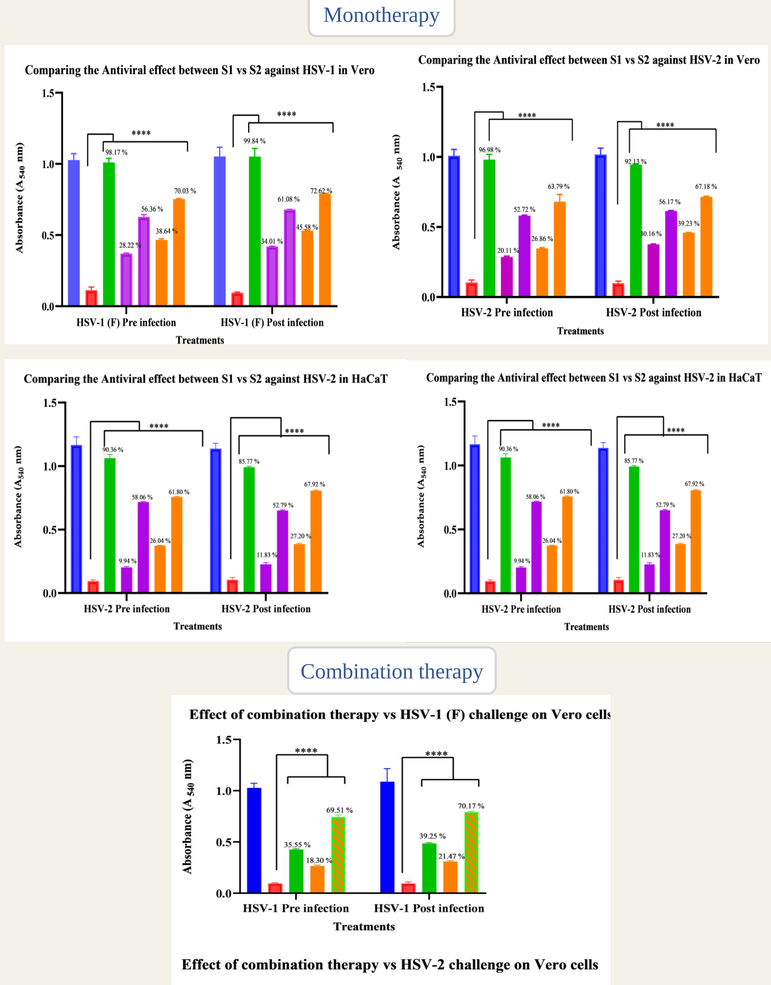


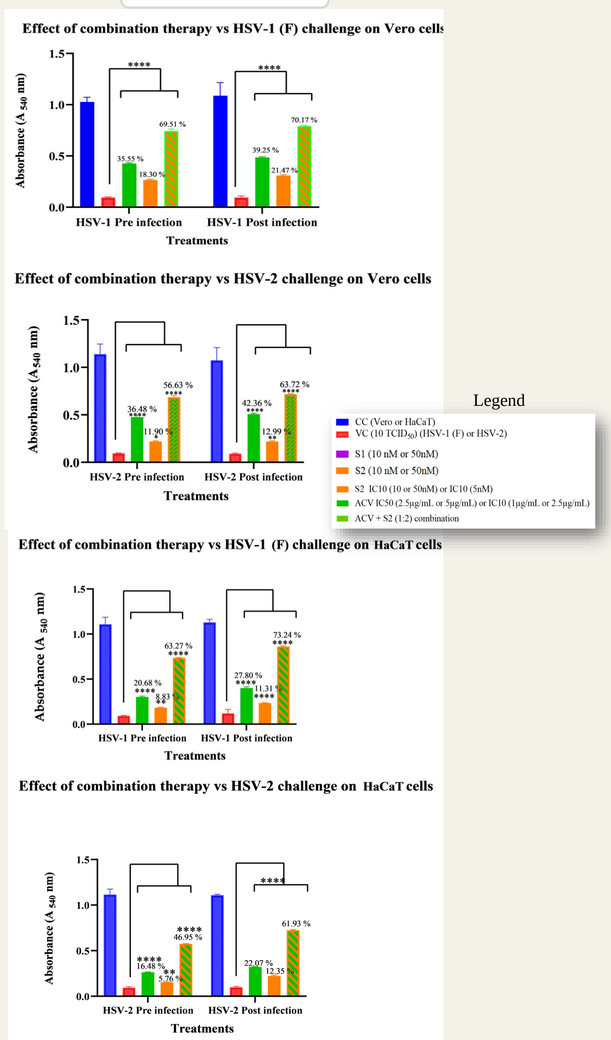
Figure 7: Brief outline of the PPI of CTTP in viral replication cycle.

Cytopathic Inhibition assay

Monotherapy



Combination therapy



Graphs 1 to 8: Representing monotherapy and combination therapy against HSV-1 and HSV-2 in Vero and HaCaT.

Results

Plaque Reduction Assay

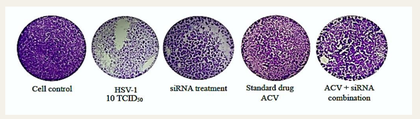


Figure 8: Plaque assay reduction in HSV-1 treated Vero cells.

	Number of Plaques (Mean ± SD)			
Treatment groups	Virus control	ACV IC10	siRNA IC10	ACV + siRNA (1:2)
Post HSV-1 infection	33 ± 2.6	11.83 ± 2.2	18.16 ± 3.54	6.16 ± 1.7
% Plaque reduction		61.29 %	51.61 %	77.42 %

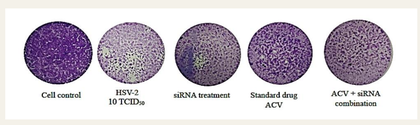
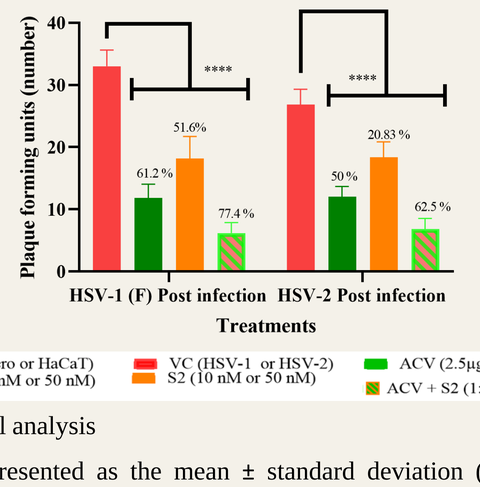


Figure 9: Plaque assay reduction in HSV-2 treated Vero cells.

	Number Of Plaques (Mean ± SD)			
Treatment Groups	Virus Control	ACV IC10	siRNA IC10	ACV + siRNA (1:2)
Post HSV-2 Infection	26.83 ± 2.48	12 ± 1.67	18.3 ± 2.5	6.8 ± 1.7
% Plaque Reduction		50.00 %	20.83 %	62.50 %

Graph 9: Plaque reduction assay in Vero cells



Statistical analysis

Data represented as the mean ± standard deviation (SD) from triplicates. Statistical differences were analysed by One-way or Two-way ANOVA with Dunnett's multiple comparisons test when appropriate.

For plaque assay each bar represents average plaque number (n=6) and analyzed by Two-way ANOVA and Dunnett's multiple comparison.

GraphPad prism v8.4 was used for analysis of results with the statistical significance set at P <0.05.

Significant values (**** P<0.0001, ***P 0.0004, **P 0.0090, *P 0.0113).

Conclusions

1) CTTP is 83% homologous in both types of HSV and highly orthologous to VZV(ORF43).
2) Delineating the interactions of CTTP with other HSV viral PPI has contributed to better understanding of possible roles and involvement in different viral protein pathways.
3) The goal was achieved using uniquely designed siRNAs capable to silence the viral encapsidation gene UL17 (CTTP).
4) *In vitro* evaluation has proved the target siRNA was non-toxic in host cell model when tested at higher doses.
5) The developed siRNA has offered consistent protection of 30-70% within 5-50nM dose range against viral challenge dose of 10TCID$_{50}$ in various in vitro techniques.
6) This work has offered new opportunities to apply RNAi as a genetic tool for anti-HSV therapy.

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Keywords: RNAi therapy, viral encapsidation, HSV, antiviral activity, Acyclovir

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