Multiple gene targeting siRNAs for down regulation of Immediate Early-2 (Ie2) and DNA polymerase genes mediated inhibition of novel rat Cytomegalovirus (strain All-03)

ABSTRACT

Background: Cytomegalovirus (CMV) is an opportunistic pathogen that causes severe complications in congenitally infected newborns and non-immunocompetent individuals. Developing an effective vaccine is a major public health priority and current drugs are fronting resistance and side effects on recipients. In the present study, with the aim of exploring new strategies to counteract CMV replication, several anti-CMV siRNAs targeting IE2 and DNA polymerase gene regions were characterized and used as in combinations for antiviral therapy. **Methods**: The rat embryo fibroblast (REF) cells were transfected with multi siRNA before infecting with CMV strain ALL-03. Viral growth inhibition was measured by tissue culture infectious dose (TCID50), cytopathic effect (CPE) and droplet digital PCR (ddPCR) while IE2 and DNA polymerase gene knockdown was determined by real-time PCR. Ganciclovir was deployed as a control to benchmark the efficacy of antiviral activities of respective individual siRNAs. **Results**: There was no significant cytotoxicity encountered for all the combinations of siRNAs on REF cells analyzed by MTT colorimetric assay ($P > 0.05$). Cytopathic effects (CPE) in cells infected by RCMV ALL-03 had developed significantly less and at much slower rate compared to control group. The expression of targeted genes was downregulated successfully resulted in significant reduction ($P < 0.05$) of viral mRNA and DNA copies (dpb + dpc: 79%, 68%; dpb + ie2b: 68%, 60%; dpb + dpc + ie2b: 48%, 42%). Flow cytometry analysis showed a greater percentage of viable and early apoptosis of combined siRNAs-treated cells compared to control group. Notably, the siRNAs targeting gene regions were sequenced and mutations were not encountered, thereby avoiding the formation of mutant with potential resistant viruses. **Conclusions**: In conclusion. The study demonstrated a tremendous promise of innovative approach with the deployment of combined siRNAs targeting at several genes simultaneously with the aim to control CMV replication in host cells.

Keyword: Cytomegalovirus; Combinations; Small interfering RNA; Replication; Gene expression