COMPLETE INHIBITION OF HEPATITIS C VIRUS RNA REPLICATION IN AN INTERFERON RESISTANT REPLICON CELL LINE BY MULTIPLE SIRNAS DELIVERED BY NANOSOMES.


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Introduction: Interferon (IFN) alpha is the standard therapy for chronic hepatitis C virus (HCV) infection but the majority of patients cannot clear virus infection by this regimen. To understand the mechanisms of resistance, we have isolated replicon cell clones and shown that defect in Jak-Stat pathway in these replicon cells lead to interferon resistance. Aim: To develop an intracellular treatment approach that can completely inhibit HCV replication and overcome IFN resistance in the cell culture model. Methods: Multiple siRNAs targeted to the IRES region of JFH-1 clone (HCV genotype 2a) was synthesized and encapsulated into biodegradable liposome based nanoparticles (nanosomes) for effective intracellular delivery. To eliminate virus replication by degradation of viral RNA in an IFN resistant GFP replicon cells was examined by cell colony assay, GFP expression by flow analysis and detection of viral RNA by RPA as well as by a highly sensitive RT-nested PCR as well as Real-Time PCR assay. Results: The intracellular deliveries of siRNA using nanosomes were highly efficient, non-toxic and can be delivered to 100% cells in culture. siRNAs targeting the fourth stem-loop of IRES sequences are highly effective in silencing HCV RNA replication in IFN resistant replicon cell lines. Multiple treatments with combination of siRNA are superior to single siRNA target in silencing HCV RNA replication that resulted in complete elimination of HCV RNA replication within a week. Using two different concentration of siRNA (50 pmole and 100 pmole) we show that GFP expression was decreased in a dose dependent manner (80% to 0.8%) within a week period. Appearance of escape mutant viruses and resistant cell colonies was not developed in cell culture when treated with siRNA-nanosomes in combination. We have developed cured cells by eliminating HCV RNA replication from IFN resistant HCV cultures by prolonged treatment with siRNA-nanosomes and HCV RNA in these cells was not detectable by using the highly sensitive RT-nested PCR assay. Conclusion: We have developed siRNA-nanosome formulations that are non-toxic and can effectively inhibit HCV RNA replication to a completion in IFN resistant replicon cells by minimizing the escape mutant development. This siRNA-nanosome formulation can be used to optimize in vivo delivery system to effectively inhibit HCV replication in the liver. Acknowledgements: This work is supported by grant from the National Institute of Health CA127481 and CA129776 and funds received from Louisiana Cancer Research Consortium, New Orleans.