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Nanosomes containing multiple siRNAs effectively inhibits HCV RNA replication – An in vitro and in vivo approach

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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: In this study, multiple siRNAs targeted to the IRES region of JFH-1 clone was synthesized and encapsulated into biodegradable nanosomes for effective intracellular delivery. The success of this approach 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 assay.

Results: The intracellular delivery of siRNA using nanosomes are highly efficient, non-toxic and can be delivered to 100% cells in culture. We show that 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.

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Biography

Dr. Sidhartha Hazari has completed his Ph.D from All India Institute of Medical Sciences, New Delhi, India and postdoctoral studies from Tulane University School of Medicine, USA. He is also obtained his DVM in Veterinary Science from Orissa Agriculture and Technology, India. He is now the senior scientist in Hepatitis Research laboratory in the Department of Pathology, Tulane University Health Sciences center. His main research focus is hepatitis C virus pathogenesis, antiviral mechanism, drug resistance, animal model development for different cancer and therapeutics. He has published more than 25 papers in reputed journals and serving as an editorial board member of repute.