ABSTRACT

Chronic liver infection by hepatitis B virus (HBV) may lead to devastating clinical conditions that include hepatocellular carcinoma and cirrhosis. Approved antiHBV drugs do not completely eradicate the infection, leading to continued viral persistence in infected individuals. Inhibition of HBV replication using synthetic activators of RNA interference (RNAi) may provide a feasible strategy of developing superior antiviral drugs. The aim of this study was to evaluate the therapeutic utility of novel 2'-*O*-guanidinopropyl [1] modified synthetic small interfering RNAs (siRNAs) to counter HBV replication in cultured mammalian cells and mice. Initially, single GP moieties were placed at different nucleotide positions of the guide strand of a potent antiHBV siRNA. Some GP-modified siRNAs enhanced antiHBV activity *in vitro* following transient transfection of Human hepatoma 7 (Huh7) cells with siRNAs and pCH-9/3091, a replication competent HBV target plasmid. These siRNAs inhibited the secretion of Hepatitis B surface antigen (HBsAg) by up to 95% in Huh7 cells. The level of knockdown exhibited by some modified siRNAs was statistically significant relative to that displayed by unmodified siRNA3 which achieved HBsAg silencing of 73%. Additionally, modified siRNAs were also capable of reducing RNA containing the *X* sequence *in vitro* by 88-93%. Impressively, some of these knockdown levels were statistically significant when compared to unmodified siRNA3, which achieved *HBx* knockdown of 83%. Quantitation of interferon (IFN) response genes by reverse transcription quantitative polymerase chain reaction (RT-qPCR) and evaluation of cell viability by 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay revealed no evidence of innate immune stimulation or cytotoxicity in cultured cells, respectively.

Abstract

Modified siRNAs also displayed moderate stability in 80% foetal calf serum (FCS). Target specificity was markedly improved by GP-modified siRNAs, especially those with seed modifications (comprising nucleotide position 2 to 8 from the 5' end of the guide strand). The siRNA-mediated mRNA cleavage product was detected from transfected cells using 5' Rapid Amplification of cDNA ends (5' RACE).

In the hydrodynamic mouse model, co-injection of GP-modified siRNAs and HBV plasmid vector led to HBsAg suppression of approximately 80-92% at day 3 and 77-96% at day 5 post-administration. The HBV knockdown levels observed at day 3 were statistically significant when compared to those displayed by unmodified siRNA3 which achieved HBsAg silencing of 58% during the same time frame. Furthermore, both sets of siRNAs also suppressed the number of circulating viral particle equivalents (VPEs) by 88-90% at day 3 post-injection. HBV silencing efficacy of 70-75% and 65% was achieved by modified and unmodified siRNAs, respectively at day 5 post-administration.

Finally, antiHBV efficacy of GP-modified siRNAs was tested in HBV transgenic mice following delivery of these RNAi effectors using cationic polyglutamate (PG) adjuvant liposomes. Both groups of antiHBV siRNAs effected HBsAg knockdown that ranged from 70-86% at day 3 to 7 post-administration as siRNA lipoplexes in HBV transgenic mice. In contrast to the unmodified siRNA3, GP-containing siRNAs achieved durable HBsAg silencing of 70% at day 14 post-administration, while the unmodified siRNA3 displayed a shorter duration of activity. As with HBsAg data, the GP-modified siRNAs also displayed silencing efficacy that was similar to the unmodified siRNA, reducing the number of

circulating VPEs by 95% from day 3 to 7 post-injection. However, the unmodified siRNA3 lost efficacy by day 14 post-administration, while the GP-modified siRNAs displayed prolonged suppression by reducing the number of circulating VPEs by 75% during the same time interval. Intrahepatic RNA levels were also assessed in transgenic mice, in which GP3 siRNA3 significantly suppressed *surface* and *core* RNA levels by 40 and 42%, respectively at day 18 post-injection. The unmodified siRNA3 suppressed *surface* RNA levels by 20% and *core* RNA levels by 25% at day 21 post-administration. Furthermore, GP4 siRNA3 silenced both *surface* and *core* RNA levels by 42% during the same time period. Additionally, intrahepatic RNA quantitation revealed no induction of IFN response genes by either unmodified or GP-modified siRNAs. In contrast to mice that had received GP-modified siRNAs, significant induction of proinflammatory cytokine release was observed in mice treated with unmodified siRNAs. The siRNA-mediated mRNA cleavage product was also detected from liver samples following 5' RACE analysis. Neither GP-modified nor unmodified siRNAs significantly induced toxicity in injected mice. Collectively, our data provide evidence that utilisation of GP-modified siRNAs and an efficient hepatotropic non-viral delivery system may be used as a strategy to counter chronic HBV infection.