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Therapeutic Silencing of MicroRNA-122 in Primates with Chronic Hepatitis C Virus Infection

Robert E. Lanford, Elisabeth S. Hildebrandt-Eriksen, Andreas Petri, Robert Persson, Morten Lindow, Martin E. Munk, Sakari Kauppinen, Henrik Ørum

The liver-expressed microRNA-122 (miR-122) is essential for hepatitis C virus (HCV) RNA accumulation in cultured liver cells, but its potential as a target for antiviral intervention has not been assessed. We found that treatment of chronically infected chimpanzees with a locked nucleic acid (LNA)–modified oligonucleotide (SPC3649) complementary to the 5′ end of miR-122 leads to long-lasting suppression of HCV viremia, with no evidence of viral resistance or side effects in the treated animals. Furthermore, transcriptome and histological analyses of liver biopsies demonstrated derepression of target mRNAs with miR-122 seed sites, down-regulation of interferon-regulated genes, and improvement of HCV-induced liver pathology. The prolonged virological response to SPC3649 treatment without HCV rebound holds promise of a new antiviral therapy with a high barrier to resistance.

Previously, we reported on potent and specific miR-122 silencing in vivo using a locked nucleic acid (LNA)–modified phosphorothioate oligonucleotide (SPC3649) complementary to the 5′ end of miR-122, which led to long-lasting decrease of serum cholesterol in mice and African green monkeys (6). Here, we investigated the potential of miR-122 antagonism by SPC3649 as a new anti-HCV therapy in chronically infected chimpanzees (genotype 1). Baseline measurements were obtained from four chimpanzees for 4 weeks, the last two of which included an intravenous (i.v.) placebo dose of saline. Two animals each were assigned to the high- and low-dose groups (5 mg kg⁻¹ and 1 mg kg⁻¹, respectively) and were treated with i.v. injections of SPC3649 on a weekly basis for 12 weeks (Fig. 1A), followed by a treatment-free period of 17 weeks. In the high-dose group, a significant decline of HCV RNA in the serum was detected 3 weeks after the onset of SPC3649 dosing, with a maximum decrease of 2.6 orders of magnitude in HCV RNA levels 2 weeks after end of treatment (Fig. 1A). Analysis of HCV RNA levels in the liver showed a decrease of 2.3 orders of magnitude in the high-dose animals. One low-dose animal achieved a viral decline of 1.3 orders of magnitude; the other experienced fluctuations in HCV RNA levels during dosing that made evaluation of the degree of suppression difficult (Fig. 1A).

We next assessed the in vivo antagonism of miR-122 in chimpanzee liver biopsies. Mature miR-122 was detected in the baseline samples (week –4) from all animals, whereas SPC3649 was detected in RNA samples obtained during treatment and up to 8 weeks after the last dose in the high-dose animals. This coincided with sequestration of miR-122 in a heteroduplex with
Fig. 1. Silencing of miR-122 by SPC3649 in chimpanzees with chronic hepatitis C virus infection. (A) Analysis of HCV RNA levels in HCV-infected chimpanzees during the study. The HCV levels are shown as genomic equivalents (GE) for the high-dose animals (4x0513, blue triangles; 4x0514, magenta diamonds) and low-dose animals (4x0267, orange squares; 4x0358, red dots) in serum (GE/ml, solid lines) and liver (GE/µg liver RNA, dashed lines). The placebo and active treatment periods are indicated below. (B) Northern blot analysis of RNA from chimpanzee liver biopsies using LNA-modified probes detecting free and sequestered miR-122 (upper panel) and SPC3649 (lower panel). The first two lanes are controls for free miR-122 and preformed miR-122/SPC3649 heteroduplexes, respectively. (C) Detection of sequence variants in the miR-122 seed sites (boxed) by deep sequencing of the HCV 5’ NCR from the high-dose animals at four time points: baseline, end of treatment, viral rebound, and end of the follow-up period. (D) The two miR-122 seed sites (boxed) in the HCV 5’ NCR are conserved in all HCV genotypes and subtypes (see Supporting Online Material for details).
We also examined the expression data for changes related to prolonged decrease in viral RNA during SPC3649 therapy. A supervised analysis of chimpanzee interferon-regulated genes (IRGs) (9, 10) revealed that the reduction in viremia was clearly associated with down-regulation of most IRGs in the high-dose animals and the responding low-dose animal (Fig. 2B and table S2). This correlated with the measured serum levels of the chemokine IP-10 (CXCL10), a highly induced IRG in HCV infections, which thereby provides a readily accessible biomarker of the hepatic IFN response during SPC3649 therapy (Fig. 2C). Together, these data imply that the endogenous IFN pathway in the liver is rapidly normalized in response to inhibition of HCV RNA accumulation even when therapy does not completely eradicate detectable viral RNA. Nonresponders to IFN-α-based HCV therapy have increased hepatic levels of IRG transcripts and serum IP-10 protein levels (11–17), reflecting a maximally induced and nonresponsive hepatic IFN response. The chimpanzee appears to be an extreme representative of this phenotype in human HCV patients, designated as IFN null-responders (18). Thus, our finding that treatment with SPC3649 results in normalization of IRG levels suggests that this therapy could be used to convert IFN null-responders to responders by reducing the viral load, thereby permitting the endogenous IFN pathway to reset to responsiveness.

Antagonism of miR-122 in chimpanzees by SPC3649 led to markedly lowered serum cholesterol in the high-dose group (Fig. 2D), similar to observations in mice and in African green monkeys (6, 19). One of the high-dose animals had a maximum decline of 44% at week 14, whereas the other animal showed a 29% decrease in cholesterol at the same time point. Pronounced decreases were observed in both low-density lipoprotein (LDL) (25 to 54%) and apolipoprotein apo-B, its primary lipoprotein component (23 to 42%) (fig. S12). In contrast to our previous findings in monkeys, where decreases in high-density lipoprotein (HDL) and its major apolipoprotein apo-A1 were more pronounced relative to LDL and apo-B (7), the observed changes in HDL or apo-A1 in chimpanzees were more variable (fig. S13). Thus, it is possible that the cholesterol-lowering effect of miR-122 antagonism is different in chimpanzees and may better reflect the expected response in humans.

To assess the safety of miR-122 antagonism after prolonged treatment with SPC3649, we monitored an extensive set of clinical chemistries and correlated them with plasma levels of the compound. The peak plasma concentrations (C_{max}) were dose-proportional and similar after first and last dose, ranging from 6.1 to 6.3 μg/ml for the low-dose animals and from 17.7 to 30.6 μg/ml for the high-dose animals (Table 1). The terminal plasma half-life was about 20 days in the high-dose animals. The plasma trough levels at the high dose ranged from 31 to 67 ng/ml and were maintained at this level for 4 weeks after the last dose (Fig. 3A). Complete blood counts, blood chemistry, coagulation markers, urinalysis, and complement activation were determined throughout the study, as were lymphocyte subsets, circulating cytokine-chemokine profiles, and additional safety parameters (table S3). No SPC3649-related abnormalities were observed for any of the measurements (Fig. 3, B and C, figs. S14 and S15, Figs. 2, 3, and 4).
Fig. 3. Treatment of HCV-infected chimpanzees with SPC3649 was well tolerated. (A) Plasma trough levels of SPC3649. (B and C) Alanine aminotransferase (ALT) levels (B) and creatinine levels (C) in HCV-infected chimpanzees during the study. (D to G) Photomicrographs of hematoxylin and eosin-stained sections from biopsies of a normal chimpanzee liver (D) and animal 4x0513 at week -4 (E), week 19 (F), and week 25 (G), respectively.

and table S3). A spike in alanine aminotransferase (ALT) was observed in one high-dose animal (4x0514), but this commenced prior to the first dose and resolved in the early dosing phase (Fig. 3B). Notably, during therapy ALT was reduced to normal levels, likely due to reduction in the viral load, and was again elevated at the end of the follow-up period when viremia returned to baseline. Histology examinations of the baseline liver biopsies from the high-dose animals revealed HCV-specific changes, including mild hepatocellular swelling with disruption of hepatocellular sinuses and cords (Fig. 3, D to G, and fig. S16). Improved liver histology was observed in both high-dose animals after treatment at week 19, indicating a response to prolonged suppression of viremia and normalization of the IFN pathway.

Our results show that antagonism of miR-122 by the LNA oligonucleotide SPC3649 leads to marked suppression of viremia in chronically HCV-infected chimpanzees, thus implying that miR-122 is essential for accumulation of HCV RNA in vivo. The good PK properties, safety profile, and high stability of SPC3649 in vivo, combined with the prolonged suppression of viremia beyond treatment, suggest that less frequent dosing could be used after viral suppression is attained. SPC3649 therapy provided a high barrier to resistance, as shown by the lack of rebound in viremia during the 12-week treatment and the lack of adaptive mutations in the two miR-122 seed sites of HCV 5′NCR. Conservation of both miR-122 seed sites in all HCV genotypes and subtypes suggests that such therapy will be genotype-independent. Finally, this study demonstrates the feasibility and safety of prolonged administration of a LNA oligonucleotide drug that antagonizes the function of a specific microRNA in a highly relevant disease model.

Table 1. Pharmacokinetic properties of SPC3649 in chimpanzee plasma. C_{max}, maximum observed plasma concentration; AUC_{total}, area under concentration versus time curve from time 0 to infinity; V_{d}, apparent volume of distribution during the terminal phase; Cl, total body clearance. Data are from week 11.

<table>
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<tr>
<th>Animal</th>
<th>C_{max} (µg ml^{-1})</th>
<th>AUC_{total} (hour · µg ml^{-1})</th>
<th>Terminal half-life (days)</th>
<th>V_{d} (liter kg^{-1})</th>
<th>Cl (ml hour^{-1} kg^{-1})</th>
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<td>22.6</td>
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References and Notes
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Supporting Online Material
www.sciencemag.org/cgi/content/full/1178178/DC1
Materials and Methods
Figs. S1 to S16
Tables S1 to S4
References
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