Direct-acting antiviral therapy for chronic hepatitis C

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Dynamic changes in HCV RNA levels and viral quasispecies in a patient with chronic hepatitis C after telaprevir-based treatment

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WHY THIS CASE IS IMPORTANT

The natural history of hepatitis C virus (HCV) indicates that acute HCV infection leads to chronic hepatitis C in approximately 75% of patients and resolves spontaneously in 25% of patients. Spontaneous clearance of viremia, once chronic infection has been established, is rare. Chronic HCV infection is one of the major causes of cirrhosis, hepatocellular carcinoma, and liver failure that can lead to liver transplantation or death. A sustained viral response (SVR) is achieved in approximately 45% of patients infected with genotype 1 after a 48-week course of pegylated interferon-α (peg-IFN) and ribavirin.

Telaprevir is a potent and selective inhibitor of the HCV non-structural 3 (NS3) serine protease and has been shown to rapidly reduce HCV RNA levels in patients. Patients receiving telaprevir, peg-IFN and ribavirin have significantly higher SVR rates compared to standard of care.

Importantly, in patients who fail to achieve an SVR, subsequent viral rebound will likely be associated with reduced genomic variability compared to the initially diverse viral population.

We describe here in detail a viral relapse after a telaprevir, peg-IFN and ribavirin treatment in a chronic hepatitis C patient which was followed by transient loss of detectable HCV RNA. Each period without detectable HCV RNA corresponded with a viral population bottleneck resulting in changes in viral quasispecies.

CASE DESCRIPTION

We report on a 54-year-old Caucasian man infected with HCV genotype 1b with cirrhosis, classified as Child-Pugh A. Medical history indicates repeated episodes of pneumothorax for which he received 2 blood transfusions in 1973 and 1976. No other risk factors for HCV transmission were reported. Analysis of the single nucleotide polymorphism at position rs12979860 upstream of the IL28B gene demonstrated a heterozygote C/T genotype. Antiviral therapy for his chronic hepatitis C was administered twice in 2003. The first treatment course consisted of amantadine and ribavirin, combined with 6 weeks of high-dose IFN-alpha-2b induction therapy followed by weekly peg-IFN. Baseline HCV RNA level of 381,000 IU/mL declined to levels below 615 IU/mL, but remained detectable. Due to the partial non-response, antiviral therapy was stopped after 24 weeks. During the second treatment course, peg-IFN, ribavirin and amantadine was administered. This resulted in a viral non-response with a maximum HCV RNA decline of 1 log IU/mL after 24 weeks of treatment. In May 2007, the patient received telaprevir (750 mg q8h) with pegylated interferon-alpha-2a 180 µg per week and ribavirin 1200 mg per day for 24 weeks followed by peg-IFN/ribavirin for an additional 24 weeks according the PROVE3 study protocol. Coinfection with human immunodeficiency virus or hepatitis B virus was excluded before study participation. HCV RNA became undetectable after 4 weeks of treatment and remained undetectable through week 40. At week 40, peg-IFN/ribavirin treatment was discontinued due to grade 3 laboratory side effects (gamma-glutamyltransferase 650 U/L, direct bilirubin 24 µmol/L, platelets 37 G/L). This patient experienced a viral relapse 4 weeks after the end of dosing and HCV RNA levels became undetectable after 4 weeks of treatment and remained undetectable through week 40.
continued to increase through week 14 of follow-up, up to a maximum viral load of 150,000 IU/mL (hereafter, ‘first relapse’; Figure 1). Subsequently, 12 weeks later (26 weeks post-end of treatment), HCV RNA could no longer be detected and remained negative thereafter for 3 additional time points, through 54 weeks (80 weeks post-end of treatment). At this time point, it appeared as though this patient experienced a spontaneous viral clearance since HCV RNA was undetectable for more than 1 year. During this period no additional infections were reported and no additional treatment was administered. Serum alanine transaminases increased at time of relapse and normalized when HCV RNA became undetectable. The grade 3 laboratory values returned to baseline levels within a few weeks after therapy cessation. Thereafter, HCV RNA was determined regularly and became positive again (<1000 IU/mL) at week 170 (130 weeks post-end of treatment; hereafter ‘second relapse’). At week 182, HCV RNA levels increased to 6280 IU/mL. No risk factors for an HCV re-infection were reported. Clonal sequence analysis was performed at 44, 54, and 182 weeks after the beginning of telaprevir-based treatment as per the method described previously. Briefly, RNA was isolated from plasma and reverse transcribed with poly-A primer. After nested PCR amplification, a 109 kb target amplicon was electrophoresed, gel-purified, and cloned into a pCR®-XL-TOPO® vector (Invitrogen, CA, USA). Escherichia coli were transformed, cultured on selective agar, and sent to Beckman-Coulter Genomics (Agencourt, MA) where the first 600 5' bases of the NS3 protease region were sequenced bidirectionally. Clonal sequence analysis revealed the presence of only V36A variants (132/132 clones) at the first relapse (post-treatment time points Weeks 44 and 54), compared to 100% wild type virus at baseline. Sequence analysis of the second relapse (Week 182) revealed that the resistance associated V36A variants were completely replaced by wild type virus (Figure 1). Diversity was evaluated by comparing the mean pairwise nucleotide and amino acid differences between each clone at each time point. Surprisingly, the phylogeny suggests that the second relapse (Week 182) did not result from outgrowth of the same viral population that had been present after the first relapse (Week 44/54; Figure 2). It appears that the first and second relapse viral populations originated from different lineages, both of which must have both persisted from before treatment. At the time of the second relapse, the NS3/4A region experienced an increase in nucleotide diversity relative to the viral population at the first relapse, but still had less genomic diversity than present at baseline (Table 1). Several studies suggested a dominant role of human leukocyte antigen (HLA) B27 in mediating viral clearance as well as viral evolution in HCV infection. HLA typing showed that this patient was HLA-B27-negative.

OTHER SIMILAR AND CONTRASTING CASES IN THE LITERATURE

Data from other telaprevir clinical trials indicate the anomalous nature of this event. Interestingly, this case report is the only reported instance of a temporarily viral clearance after treatment failure in a telaprevir containing regimen, although the phenomenon of viral diversity reduction may occur commonly after treatment failure in patients that achieve undetectable HCV RNA. No patients were reported to have had a similar undetectable HCV RNA period after a long-term follow-up study (EXTEND study) of 79 patients after failing to achieve an SVR with telaprevir-based treatment.12

Figure 1. Course of HCV RNA, alanine amino transferase (ALT) and NS3/4A clonal analysis during treatment and follow-up. See page 285 for full colour figure.
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DISCUSSION

We report here a case of a viral relapse following combination therapy with telaprevir, peg-IFN and ribavirin resulting in a viral population consisting of only V36A resistance associated variants. Subsequently, this patient had a transient disappearance of HCV RNA for more than 1 year in the absence of antiviral therapy. Thereafter, HCV RNA reappeared again with a viral population consisting of only wild type virus. A genetic bottleneck induced by telaprevir, peg-IFN and ribavirin treatment resulted in a viral population at the first relapse (Weeks 44 and 54) with less diversity than before treatment. We hypothesize that the reduced heterogeneity resulted in a viral population more sensitive to the host immune response than the parental population. The HCV genome in single hosts is a dynamic population of different but closely related genomes. At baseline, on average, each clone from this genotype 1b patient was 2.8% different from each other clone. By Weeks 44 and 54, after relapse, the average percent difference between clones had been reduced to only 0.7% (Table 1).

Figure 2. Phylogeny of clonal sequences. Black lines denote clones (n = 76) sequenced from the pre-treatment time point.

Consistent with general population biology theory, it could be expected that this reduction in diversity resulted in a viral population less capable of adapting to changes in its environment. We propose that this genetic bottlenecking may occur whenever a treatment regimen produces an extremely significant reduction in viral load. As the viral population continues to evolve after the initial viral rebound event, the viral population may continue to adapt and return to the pre-treatment level of diversity after an indeterminate period of time. Consistent with this hypothesis, by Week 182, the viral population which was 100% wild type, had greater nucleotide diversity than the Week 44/54 population, encoding as much amino acid diversity as was present at baseline. Longitudinal sampling of the diversity of viral populations in numerous patients that fail treatment regimens after experiencing reductions of viral load to below 25 IU/mL would be necessary to confirm, or refute, this hypothesis. It is interesting and surprising that the viral population at the second relapse (Week 182) does not appear to represent a continuation of the viral lineage that was present at the first relapse (Week 44/54; Figure 2). The phylogeny suggests that the second relapse population originated from a different fraction of the parental (baseline) quasispecies than the first relapse population. This observation, together with the absence of risk factors for HCV transmission, suggests that re-infection in this patient is highly unlikely.

In conclusion, treatment with telaprevir, peg-IFN and ribavirin appears to have resulted in a genetic bottleneck leading to a reduction of variability in the hepatitis C viral population. This reduction in viral variability could potentially lead to a reduced viral capacity to adapt to changes in the host, including immune responses. In this single case, we hypothesize that the adaptive host immune response could have lead to the clearance of the majority of viral population observed at relapse but that a minority population present at undetectable levels at that time point was not cleared, leading to a second outgrowth of virus. Patterns of significant genomic diversity reduction during treatment with direct-acting antivirals may represent a more broadly applicable phenomenon. Long term follow-up of patients who fail to respond to a combination regimen including a direct-acting antiviral agent is needed to further study these phenomena.

The portions of the tree drawn in blue and green represent the monophyletic clades recovered at Week 44/54 (n = 78) and Week 182 (n = 90), respectively. The maximum likelihood phylogeny, created with PhyML, assumes a general-time reversible model of nucleotide substitution using empirical estimations of both invariance and the alpha parameter of the gamma distribution, with the confidence of nodes assessed with 100 replicates of the bootstrap. The tree is drawn rooted in the node which differentiates the lineages of the Week 44/54 and Week 182 viral population with 99% bootstrap support. Internal nodes basal to the Week 44/54 and Week 182 viral populations with greater than 50% bootstrap support are labeled.
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REFERENCES