Naturally occurring hepatitis C virus (HCV) NS3/4A protease inhibitor resistance-related mutations in HCV genotype 1-infected subjects in Italy

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Objectives: To assess the prevalence of hepatitis C virus (HCV) NS3/4A protease inhibitor (PI) resistance mutations in HCV genotype 1-infected PI-naive individuals in Italy.

Patients and methods: One hundred and twelve patients infected with HCV genotype 1a or 1b (based on Versant HCV Genotype 2.0 or 5′UTR/core sequencing) and never treated with any HCV PI were evaluated. The whole NS3 region was analysed by population sequencing and mutations related to resistance to linear and macrocyclic PIs were recorded.

Results: Forty-six HCV-monoinfected and 66 HCV/HIV-coinfected subjects were studied. Complete NS3 sequence information was obtained for 109 (97.3%) samples: 67 subtype 1a and 42 subtype 1b. Subtype assignment by NS3 sequencing was concordant in 100.0% and 83.9% of cases with the original 5′UTR sequencing and Versant result, respectively. At least one mutation related to PI resistance was detected in 21 (19.3%) isolates. However, 11 of these had only Q80K, expected to confer resistance to one investigational macrocyclic compound, and were detected only in subtype 1a. Boceprevir and telaprevir resistance-related mutations were detected in 10 (9.2%) isolates and included V36L, T54S and V55A. Only one isolate harboured two mutations (V36L and T54S). There was no association between HCV PI resistance and HIV coinfection or exposure to HIV PIs.

Conclusions: A minority of untreated HCV genotype 1 patients in Italy harbour a virus population carrying HCV PI resistance-related mutations. The clinical implications of this finding warrant further analysis.

Keywords: PIs, drug resistance, genotyping

Introduction

The hepatitis C virus (HCV) NS3/4A protease is the target of several macrocyclic and linear inhibitors.1 Compared with the pegylated interferon plus ribavirin standard of care (SOC), the SOC plus either of the first two approved linear inhibitors, telaprevir and boceprevir, was more effective in HCV genotype 1-infected subjects.2 However, in vitro and in vivo studies have documented boceprevir/telaprevir-driven selection of unique mutations and cross-resistant mutations in the NS3 protease catalytic domain.3 This is leading to a paradigm shift in the monitoring of HCV treatment because selection of resistant variants has not been an issue with the pegylated interferon plus ribavirin treatment. Along with the definition of HCV mutations associated with decreased susceptibility to the different protease inhibitors (PIs), sequence analysis of the HCV NS3 genome region may become an integral part of the management of patients who are under treatment or are candidates for treatment. In this study we investigated the prevalence of HCV NS3 amino acid changes provisionally included among the NS3/NS4A inhibitor resistance-associated mutations in a group of HCV-infected subjects living in Italy and not previously treated with PIs.

Patients and methods

Patients

Plasma samples were obtained between 2007 and 2011 from 112 patients attending the Clinical Infectious Diseases Unit of the Catholic
University of Rome or Infectious Diseases Unit I or II of the University Hospital of Siena. Written informed consent was obtained from each study participant. Patients were infected with HCV genotype 1a or 1b, based on sequencing of the 5’UTR plus part of the core region or the Versant HCV Genotype 2.0 assay (LiPA) (Siemens, Milan, Italy). HIV co-infection was present in 66 (58.9%) cases. At the time of sampling, all the patients had detectable plasma HCV RNA and were naïve to anti-HCV treatment with PIs.

Amplification and sequencing of the NS3 region

Viral RNA was extracted from plasma samples using the QIAamp Viral RNA Minikit (Qiagen, Hamburg, Germany). A 2383 bp DNA fragment encompassing the whole NS3/4A region (coordinates 2640–5474 of the H77 HCV genome, GenBank accession number AF009606) was obtained by random hexamer-driven reverse transcription followed by a heminested PCR. Primers P519Fwd (5′-TCCTTCTCYGTGTTCTTCG-3′) and P520Rev (5′-GCCTCTGTTGGAAAYGGCT-3′) were used in the first 30-cycle PCR and primers P523Rev (5′-GCACTYTCATCCTCACGAA-3′) and P519Fwd were used in the second 35-cycle PCR. Bidirectional DNA sequencing was performed using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Life Technologies Corporation, Carlsbad, CA, USA) with five different primers spanning the whole NS3 region: P525Fwd (5′-CGGTCGCTYCCGGAWG-3′), P529Rev (5′-TRGTGCTCTTCGCGTCCG-3′), P528Rev (5′-AGRTGYCTCCCCCTGTGAT-3′), P527Fwd (5′-GACCCYACC TTYACCATTGGAC-3′) and P530Rev (5′-AGCCGGCTCATAGACCT-3′). NS3 sequences were aligned with genotype 1a, 1b and 1c reference sequences obtained from the Los Alamos HCV Sequence Database (http://hcv.lanl.gov/content/index). A phylogenetic tree was constructed by the neighbour-Joining method using the PHYLIP software package version 3.69 (http://evolution.genetics.washington.edu/phylip/html) and edited with FigTree version 1.3.1 (http://tree.bio.ed.ac.uk/software/figtree/). Sequences were submitted to GenBank under accession numbers JN704192 to JN704300.

Scoring of PI resistance mutations

In the absence of a universally accepted reference list for HCV PI resistance mutations, a comprehensive list of linear and macrocyclic PI resistance mutations was derived by combining the lists recently published by Kieffer et al., Soriano et al. and Halfon and Locarnini. Results

The median (IQR) age at testing was 48 (44–52) years and 59.8% of the patients were males. The median (IQR) HCV viral load was 6.03 (5.58–6.61) log IU/mL. NS3 amplification and sequencing was successful with 109 (97.3%) samples, the three failures not being associated with low-level viraemia (data not shown). Phylogenetic analysis identified 67 and 42 NS3 sequences as belonging to genotype 1a and 1b, respectively. HIV-coinfected patients were more likely to harbour genotype 1a than HCV-monoinfected patients (50/64 versus 17/45; P < 0.0001, Fisher’s exact test). Genotype 1a sequences clustered within two different sub-clades of similar size (Figure 1). Of note, there were 10 of 62 (16.1%) cases in which the original subtype assignment by LiPA was not confirmed by NS3 sequencing: one 1a was genotyped as 1b in NS3 and nine 1b were genotyped as 1a in NS3. By contrast, all the genotype assignments based on 5′UTR plus partial core sequencing were concordant with those obtained by NS3 sequencing.

Overall, NS3 mutations associated with resistance to boceprevir, telaprevir or macrocyclic PIs were scored in 21 (19.3%) cases (Table 1). There was no significant association between HCV PI resistance mutations and HIV coinfection. Likewise, exposure to HIV PIs in the HIV-infected patient population was not associated with HCV PI resistance mutations, detected in 9/52 (17.3%) of the HIV PI-exposed patients and in 2/12 (16.7%) of the HIV-positive HCV PI-naïve patients. The most common mutation was Q80K, included in the macrocyclic PI but not in the boceprevir and/or telaprevir resistance lists. Interestingly, this mutation was detected only in genotype 1a sequences (11/67 in genotype 1a versus 0/42 in genotype 1b; P = 0.006, Fisher’s exact test). By contrast, the other PI resistance mutations found were all related to boceprevir and telaprevir, but not macrocyclic PIs, and were evenly distributed in genotypes 1a and 1b. Only one viral strain had two resistance mutations (V36L and T54S).

Discussion

The HCV NS3 genotyping system presented was very effective, failing to amplify only 2.7% of the samples analysed. Full-length sequencing of the NS3 region may also make it possible to obtain genotype and subtype information with increased reliability with respect to hybridization assays such as LiPA. We indeed observed 16.1% of cases in which LiPA and phylogenetic analysis of the NS3 sequence yielded a different subtype; in particular, several 1b genotypes by LiPA were classified as 1a by NS3 sequencing. Complete NS3 sequencing also made it possible to discern two different lineages or sub-subtypes within genotype 1a, as recently reported in a full-genome sequence study. It is presently unknown whether this distinction has any relevance in the setting of clinical use of NS3/4A PI therapy.

Using a combined list of NS3/4A PI resistance mutations as a reference, as many as 19.3% of patients harboured a prevalent virus population with at least one NS3/4A PI resistance mutation. However, all but one case had just one mutation and half of the cases had only Q80K, so far documented to confer resistance to only one macrocyclic PI (TMC435350). Nevertheless, the strong association of this mutation with genotype 1a, reported also in another study, may discourage the use of this compound in patients infected with this HCV sub-genotype. Thus, mutations associated with resistance to the currently licensed HCV PIs boceprevir and telaprevir (V36L, T54S and V55A) were detected in only 10 (9.2%) of the patients included in this study. With respect to other reports, we found a larger percentage of patients harbouring a prevalent virus population with the V36L mutation (5.5% versus 0.9%–1.6%). Unlike our study, some reports have documented the occurrence of V36L or T54S only in HCV genotype 1a-infected patients. However, the small number of cases and the uncertainty of subtype assignment by different methods do not allow at present the establishment of significant subtype specificity for these and other naturally occurring boceprevir/telaprevir resistance mutations. It must be noted that the most important boceprevir/telaprevir resistance mutations (V36A/M, T54A, R155K, A156S/I/V and V170A) were not detected. Clearly, the question remains whether and which baseline HCV species carrying drug resistance-related mutations have a significant impact on
Figure 1. Radial and linear dendrograms of the 109 HCV NS3 sequences obtained together with reference sequences for subtypes 1a, 1b and 1c (outgroup). Reference sequences are indicated by the prefix ‘Ref.’ in the taxon label in the linear dendrogram.
response to NS3/4A inhibitor treatment. While the use of PIs is established as an SOC in HCV-infected patients, analysing the evolution of the target region in the HCV genome is warranted in order to disclose the role of HCV drug resistance and provide the most convenient virological monitoring for patients who are candidates for treatment or are under treatment.

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