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HCV RNA decline in the first 24 h exhibits high negative predictive value of sustained virologic response in HIV/HCV genotype 1 co-infected patients treated with peginterferon and ribavirin

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ABSTRACT

Background: Treatment with Peg-interferon and ribavirin (PEG-IFN/RBV) for HIV patients co-infected with hepatitis C virus (HCV) genotype 1 has suboptimal rates of response. Viral kinetics has emerged as one of the best prognostic factors of treatment outcome.
Methods: Twenty HIV/HCV genotype 1 co-infected patients in treatment with PEG-IFN/RBV, had blood drawn at baseline, 24 h, 4, 12, 24, 48, and 72 weeks. HCV-RNA levels were evaluated at each time point. ROC curves were used to evaluate the $\log_{10}$ HCV-RNA decay at 24 h that exhibits the best predictive value of achieving response. Genomic characterization of HCV NS5A at both interferon sensitivity-determining region (ISDR) and protein-kinase binding (PKRBD) domains were performed in order to evaluate its heterogeneity and association with 24 h HCV-RNA decay and SVR.
Results: Non-responder patients exhibited a mean of 0.7 $\log_{10}$ (SD 0.74 $\log_{10}$) HCV-RNA decay at 24 h, whereas responder-patients presented 1.6 $\log_{10}$ (SD 0.28 $\log_{10}$), $p = 0.04$. A reduction in HCV viral load from baseline to 24 h of <1.4 had a negative predictive value for achieving SVR of 100% and a positive predictive value of 50%. HCV genotype 1 isolates from patients with a decrease of HCV-RNA at 24 h >1.4 $\log_{10}$, exhibited 3.1(SD 1.5) amino acids substitutions in ISDR and 4.8(SD 2.3) in PKRBD regions and 1.6(SD 0.7) and 2.4(SD1.3), respectively, in those patients presenting lower reduction in HCV-RNA.
Conclusions: HIV/HCV genotype 1 co-infected patients with a decrease in HCV-VL at 24 h >1.4 $\log_{10}$ are more likely to achieve SVR when treated with PEG-IFN/RBV than those with lower levels of HCV-RNA decay. Along with other host-related and viral-related prognostic factors in HIV/HCV co-infected patients, this very early time point of evaluation could be of relevance in the management of HCV-specific treatment.

1. Introduction

Around 20% of the people living with HIV/AIDS worldwide are co-infected with HCV (Soriano et al., 2010); similar rates of co-infection have been observed in Argentina (Laufer et al., 2010).

In the context of highly active antiretroviral therapy, chronic hepatitis C has emerged as one of the leading causes of morbidity and mortality in HIV patients, mainly in developed countries (Rockstroh et al., 2005; Weber et al., 2006). The combination of pegylated interferon (PEG-IFN) and ribavirin (RBV) is the best available treatment for chronic HCV infection both in HCV monoinfected patients and in those co-infected with HIV (Chung et al., 2004). However, this treatment results in sustained virological response (SVR) in less than 50% of co-infected patients (Chung et al., 2004).

Virological response kinetics has emerged as one of the best prognostic factors of treatment outcome (Van den Eynde et al., 2009). HCV kinetics studies during treatment with PEG-IFN, showed that HCV-RNA generally declines with a biphasic pattern, consisting of a rapid first phase lasting for approximately 1–2 days, followed by a second phase less pronounced of HCV-RNA decline (Dahari et al., 2008). There is increasing evidence that early time points during treatment, such as HCV viral load at weeks 2 and 4 can be used to guide and individualize therapy in both HCV-monoinfected and HIV/HCV-co-infected patients (Neumann et al., 2009). Furthermore, a correlation has been observed between HCV-RNA decline in the first 48 h of treatment and SRV in monoinfected

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patients (Durante-Mangoni et al., 2009); however, this correlation remains controversial in the presence of HIV co-infection (Arends et al., 2009; Araújo et al., 2010).

Both host and viral factors influence the response to specific antiviral treatment (Kau et al., 2008). The main host factors that affect the rate of SVR are age, body mass index, liver fibrosis, and insulin resistance (Bortoletto et al., 2010). IL28B-associated polymorphisms are also a host factor that has been linked with response to therapy (Ge et al., 2009; Thomas et al., 2009). High baseline viremia, HCV genotype (Lindh et al., 2010) and mutation in NS5A region have been described as viral factors influencing HCV therapy outcome (Kau et al., 2008). The NS5A protein of HCV has the potential to block IFN-induced RNA-dependent protein kinase (PKR) and may therefore interfere with the response to IFN therapy. More than 4 mutations in the NS5A PKR-binding domain (PKR-BD) have been associated with responsiveness to IFN-alfa. Specifically inside the PKRBD, the Interferon Sensitivity Determining Region (ISDR) has been described to interfere with HCV viral response to IFN (El-Shamy et al., 2008; Enomoto et al., 1996).

The present analysis evaluates the predictive value for SVR of HCV viral load (HCV-VL) decline at 24 h of initiation of PEG-IFN/RBV therapy in a cohort of 20 HIV/HCV genotype 1 co-infected individuals. We have also studied the association of 24 h decay of HCV RNA with baseline host factors as well as viral characteristics including HCV genotype 1-NS5A genomic heterogeneity at the PKR-BD.

2. Methods

2.1. Patients and samples

This is a prospective cohort study of HIV patients co-infected with HCV genotype 1, from a single hospital in Buenos Aires, Argentina, treated in 2007 and 2008 with PEG-IFN and RBV. The protocol was approved by the ethics committee at the Universidad de Buenos Aires. Patients that gave their informed consent were included in the analysis if they met the following inclusion criteria: infection with HCV genotype 1, previously untreated chronic hepatitis C with PEG-IFN and ribavirin, positive HCV-RNA in plasma, ALT higher than 1.5 fold upper normal limit; CD4+ cell count above 200 cells/mm^3 and HIV viral load below 50,000 copies/mL, in response to a stable antiretroviral treatment or without antiretroviral treatment if not required by the current national guidelines. Exclusion criteria included: presence of other causes of liver disease, decompensated cirrhosis, pregnancy and potential contraindications for interferon or for ribavirin therapy like hemoglobinopathies, cardiopathy, autoimmune diseases, major depression or other severe psychiatric pathologies, as well as active drug consumption within the last twelve months. A total of 20 patients were included.

Treatment was planned for 48 weeks in all patients. Fifty percent of patients received subcutaneous PEG-IFN alfa-2b (Peg-Intron-A, Schering Corp., Kenilworth, NJ) (80 mcg–150 mcg, body weight-adjusted dosing) each week plus oral ribavirin (Rebetol, Schering Corp., Kenilworth, NJ) every day; and 50% of patients received subcutaneous PEG-IFN alfa-2a (Pegasys, Roche Corp., Hertfordshire, UK) (180 mcg) each week plus daily oral ribavirin (Copegus, Roche Corp., Hertfordshire, UK). RBV dosing was body weight-adjusted in all cases: 800 mg when the body weight was below 60 kg, 1000 mg when it was between 60 and 75 kg and 1200 mg when body weight was above 75 kg. When at least a 2 log reduction in HCV RNA at week 12 was obtained, patients continued treatment and were re-evaluated at week 24; if HCV RNA was not detectable, treatment was continued until week 48.

2.2. Monitoring

Patients were evaluated before beginning treatment, at 24 h, 2 weeks after starting therapy and every 4 weeks until the cessation of therapy, and 24 weeks after the end of treatment to evaluate SVR. Blood samples were drawn at baseline, 24 h, 4, 12, 24, 48, and 72 weeks. HCV-VL (Bayer VERSANT® HCV RNA 3.0 Assay, range 615–7,690,000 IU/mL) and HCV qualitative PCR (Cobas Amplicor HCV 2.0, lower limit of detection 50 IU/mL) were evaluated at each time point. Genotype was evaluated with Versant HCV Genotype 2.0 Assay (LiPA). Plasma samples were frozen at −80 °C until use.

2.3. RT-PCR and direct sequencing of the HCV NS5A region

HCV RNA was extracted from 200 μl of pre-treatment plasma by Trizol LS (GIBCO, Life Technologies) from 19 of the 20 baseline isolates. Isolated HCV-RNA was reverse transcribed using MMLV reverse transcriptase (Invitrogen) in the presence of NS5A antisense primer.

NS5A amplifications were performed as previously described (Bolcic et al., 2008). Amplification products were sequenced by the use of Big Dye Terminator Kit v.3.0 (Applied Biosystems) in the ABI Prism 3100 automatic sequencer (Applied Biosystems).

2.4. Sequence analysis

Sequences of all samples were edited with Sequencher software v.4.10.1 (Gene Codes) and aligned with Mafft program (http://mafft.cbrc.jp/alignment/server/). Nucleotide sequences were translated into amino acid sequences. The aminoacidic substitution number of NS5A PKRBD were visually counted and compared with the HCV genotype 1a prototype M62321.

2.5. HCV viral load analysis

The following definitions were used to analyze the HCV kinetics in the present study. Rapid virological response (RVR) was considered when HCV RNA was undetectable by a qualitative technique (lower limit of detection 50 IU/mL) at week 4. Partial early virological response (pEVR) was defined as a 2 $\log_{10}$ HCV-VL decay from baseline at week 12 of treatment, and complete early virological response (cEVR) was defined as an undetectable HCV viral load (quantitative technique, lower limit of detection 615 IU/mL) at the same week. Twenty-four weeks response, end of treatment response (ETR) and sustained viral response (SVR) were defined as undetectable HCV RNA by a qualitative technique (lower limit of detection 50 IU/mL) at weeks 24, 48 and 72, respectively. Finally, 24 h HCV RNA change was calculated as the decrease in HCV-VL from baseline at 24 h: $\Delta 24\text{–}0 = \log_{10} V24 - \log_{10} V0$. $V24$ describes the HCV viral load at 24 h and $V0$ the viral load at baseline.

2.6. Statistical analysis

A descriptive analysis of baseline variables was conducted looking at the central tendency and dispersion. These values were compared with the aim of evaluating if the demographic, epidemiological, clinical, biochemical and histopathological characteristics were similar among patients who achieved SVR and those who did not. Fisher's exact test was used to analyze qualitative variables and Mann–Whitney U test to analyze quantitative variables. The significance level was set at 5% and all tests were 2-tailed. The area under the receiving operating curve (AUROC) was used to calculate the cut-off point in viral load decline at 24 h with the best sensitivity, negative and positive predictive value.