Original article

Very early viral kinetics on interferon treatment in chronic hepatitis C virus genotype 4 infection

Wolfgang Jessner1,2, Michael Gschwantler3,4, Elisabeth Formann1,4, Colin Gurguta1,5, Thomas Watkins-Riedel6,7, Friedrich Wrba8 and Peter Ferenci*

1Department of Internal Medicine III, Gastroenterology and Hepatology, Vienna Medical University, Vienna, Austria
2Department of Internal Medicine, Gastroenterology and Hepatology, Innsbruck Medical University, Innsbruck, Austria
3Department of Internal Medicine IV, KA Rudolfstiftung, Vienna, Austria
4Present affiliation: Department of Medicine IV, Wilhelminenspital-Hospital of Vienna, Vienna, Austria
5Present affiliation: Department of Medicine I, Hospital Hietzing, Vienna, Austria
6Department of Clinical Virology, Medical University of Vienna, Austria
7Present affiliation: Department of Hygiene, Wilhelminenspital-Hospital of Vienna, Vienna, Austria
8Department of Clinical Pathology, Medical University of Vienna, Austria

*Corresponding author: E-mail: peter.ferenci@meduniwien.ac.at

Background: Interferon (IFN)-resistant hepatitis C virus strains limit efficacy of antiviral combination therapy in patients infected with genotypes 1 and 4. A single test dose of IFN was useful to identify non-responders to IFN-α2b/ribavirin (RBV) or likely non-responders to pegylated (PEG)-IFN-α2a/RBV therapy in genotype 1 patients. Our aim was to investigate this approach in genotype 4 patients.

Methods: Viral load was measured in 46 patients before and 24 h after 10 megaunits (MU) IFN-α2b, and before and during 2 weeks of daily 5 MU IFN-α2b administration. Thereafter, patients received 48 weeks combination therapy with either 180 μg PEG-IFN-α2a/RBV (n=33), 1.5 μg/kg PEG-IFN-α2b/RBV (n=7) or 5 MU IFN-α2b/2 days (n=6), along with 1–1.2g RBV/day. For prediction analysis the largest group (PEG-IFN-α2a) was evaluated only.

Results: Median 24 h log10 change after 10 MU IFN-α2b was 1.15 (range 0.08–2.48) and after 5 MU IFN-α2b was 0.81 (-0.12–2.22; P<0.0001). Log10 changes after 2 weeks on 5 MU IFN-α2b daily and 24 h after 10 MU were the best predictors of early virological response (defined by negativity of a standard qualitative PCR) to PEG-IFN-α2a/RBV combination therapy (area under curve [AUC]=0.97; P<0.001, receiver operating characteristics), 24 h log10 change after 10 MU was the best predictor of sustained virological response (SVR; AUC=0.91, P=0.001).

Conclusion: As in genotype 1 patients, there is large variation in IFN responsiveness, including the presence of resistant strains, in genotype 4 patients. A 24 h log10 change after 10 MU IFN-α2b is an excellent predictor of SVR on PEG-IFNα2a/RBV combination therapy. This test may be useful to obtain homogeneous groups for clinical studies and could help in clinical decision making.

Introduction

For hepatitis C virus (HCV), at least six different genotypes have been described [1]. Although genotype-specific differences in the natural course of chronic infection have not yet been clearly worked out, large trials have shown the significance of genotypes as independent predictors for response to antiviral therapy [2–5]. Genotype 2 and 3 patients require 24 weeks of combination therapy only [5], whereas most patients with genotype 1 need treatment for at least 48 weeks.

Genotype 4 is common in Egypt, Central Africa and in Middle East countries, such as Saudi Arabia [6], Bahrain, Yemen and Iran. In Egypt, the overall prevalence of anti-HCV antibodies is about 20% and reaches up to 60% in some age groups in high-prevalence areas [7]. This is thought to be mostly a result of intravenous antischistosomal therapy under insufficient hygienic conditions not stopping before 1986, when the oral agent praziquantel was introduced [7]. However, in the USA and Europe, genotype 4 prevalence is low, but prevalence appears to be on the rise [8] because of intravenous drug abuse at a young age [9,10]. Higher prevalences have been reported in
some areas such as southern Italy [11] and southern Spain [12]. Overall, efficacy of interferon (IFN)/ribavirin (RBV) combination therapy in genotype 4 patients is somewhere between genotype 1 and 3 with sustained virological response (SVR) rates of 33–70% [5,8,13–16]. In trials assessing the optimal duration of therapy in HCV genotype 4 patients, 36 or 48 weeks were superior to 24 weeks [17–19]. Thus, in terms of demanding similar amounts of treatment as in genotype 1 infection, genotype 4 patients represent a difficult-to-treat population despite improved SVR rates.

As shown in a number of studies, there is a highly variable sensitivity to IFN in genotype 1 infection. This is reflected by viral kinetics in the first 2–4 weeks on therapy and even by the 24 h log10 change after a single standard IFN dose, which may vary between 0 and at least 2 logs among patients [20–26]. These kinetic parameters have high predictive power for outcome of standard IFN monotherapy as well as standard and pegylated (PEG)-IFN combination therapy. The aim of the present study was to examine viral kinetics and prediction of treatment response in genotype 4 patients and to compare IFN sensitivity parameters with previous results in genotype 1 patients.

### Methods

#### Patients

Forty-six consecutive IFN-naive patients chronically infected by HCV genotype 4 were enrolled in two Austrian centres from April 2000 to March 2003. Forty-one patients (89%) were immigrants from Egypt. Of these, 30 patients (73.2%) showed evidence for previous schistosomiasal infection by serology. Nineteen patients reported on having received parenteral treatment for schistosomiasis in Egypt and three for other infections. Five patients were of Austrian origin (11%) – three were former intravenous drug abusers and another repeatedly had sexual contact with an intravenous drug abuser. No possible route of infection could be identified in the remaining Austrian patient.

Standard criteria were used for patient selection [2–4]. In particular, patients with evidence for liver diseases other than hepatitis C, active substance abuse, coinfection with hepatitis B virus or HIV, or significant psychiatric disease were excluded from the study.

#### Protocol

HCV viral kinetics were studied in patients during 3 weeks on standard IFN monotherapy before initiation of combination therapy as described in a previously published study on genotype 1 patients [20]. Log10 changes in virus concentration were used as parameters for IFN sensitivity.

On day -21, patients received a single dose of 10 MU IFN-α2b (IntronA® AESCA-Schering Plough Co., Traiskirchen, Austria). Starting 1 week later (day -14), patients were given 5 MU IFN-α2b daily for the following 2 weeks (days -14 - -20), before and exactly 24 h after the first 5 MU dose (days -14 and -13), and on days -7 and -1. The 10 MU and first 5 MU IFN injections were given during the morning by the physician in the clinic, the following daily injections were done by the patients in the evening. On day 0, combination therapy was started using 180 μg PEG-IFN-α2a (Pegasys®, Roche, Vienna, Austria) weekly plus 1–1.2 g RBV (Copegus®, Roche) in 33/46 patients. The remaining patients received alternative treatments of either 5 MU IFN-α2b every other day (n=6) or 1.5 μg/kg PEG-IFN-α2b weekly (PegIntron® AESCA-Schering Plough Co., Traiskirchen, Austria) (n=7), plus 1–1.2 g RBV daily (Rebetol®, AESCA-Schering Plough Co.). In all three groups, treatment was given for 48 weeks in case of early virological response (EVR).

The choice of the respective IFN preparation reflected the availability of the drugs in Austria and our research interests [26]. The aim of this study was not to evaluate possible differences in efficacy among the IFN preparations. All 46 patients were included in the analysis to compare genotype 4 kinetics with a historical genotype 1 sample (as reported in the Discussion). The analysis of outcome prediction after combination therapy in genotype 4 patients was done only for the 33 patients treated with 180 μg PEG-IFN-α2a/RBV. The protocol was approved by the ethics committees of both participating hospitals.

#### Definition of virological response

Treatment response was assessed at various time points: at week 12 (after 2 weeks of monotherapy and 12 weeks of combination therapy) by qualitative and quantitative PCR, and at week 24 (after 2 weeks of monotherapy and 24 weeks of combination therapy), at end of treatment (EOT) and at 24 weeks after EOT by qualitative PCR.

The following on-treatment (at week 12 of combination therapy) response criteria were applied [2,3]: EVR was defined as undetectable HCV by qualitative PCR; slow virological response was defined as a 2 log10 decrease in viral load, but detectable HCV; and non-response was defined as <2 log10 drop in viral load. End of treatment (EOT) virological response was defined as undetectable HCV by qualitative PCR at the end of treatment. SVR was defined as undetectable HCV by qualitative PCR 24 weeks after end of treatment.
Based on our experience in patients with genotype 1 HCV [20], we assumed that the 2 week course of standard IFN monotherapy does not influence the outcome of PEG-IFN-α2a/RBV combination therapy.

Determinations

Quantitative testing

Viral load was determined using the COBAS AMPLICOR HCV MONITOR™ test, v2.0 (Roche Diagnostic Systems, Branchburg, NJ, USA) following the manufacturer’s guidelines. Samples with viral load >500,000 IU/ml were 1:100 diluted and retested. Analysis included undiluted values only to avoid artificial high log10 changes caused by dilution of one sample only [27]. Data are expressed as IU/ml. Quantitative limit of detection is between 200–600 IU/ml (corresponding to about 400–1,000 copies/ml) depending on test performance. The coefficient of variation of the test was 11% in the high-titre range (2.1×10^4–1.9×10^5 IU/ml; 95% confidence limit) and 13% in the low-titre range (1.3×10^4–1.2×10^4 IU/ml). Positive controls were tested in triplicate within 20 consecutive runs for determination of pooled intra- and interassay coefficients of variation.

Qualitative testing

HCV PCR was determined by COBAS AMPLICOR® HCV test, v2.0 (Roche Diagnostic Systems) following the manufacturer’s guidelines. The lower level of 100% detection of HCV RNA is 50 IU/ml (~100 copies/ml).

Genotyping

HCV genotypes were determined by the Line Probe assay (Innogenetics N.V. Zwijnaarde, Belgium). In addition, genotypes and subtypes were determined by amplification and sequencing of a 255 base-pair fragment in the NS5B region. The sequences were aligned and genotype/subtype determinations were performed by means of phylogenetic analysis, including reference sequences from non-genotype 4 HCV and various genotype 4 HCV subtypes [28].

Statistical analysis

All viral load data are log10-transformed and given in IU/ml. Groups were compared by Mann–Whitney U test unless otherwise specified. A stepwise forward logistic regression analysis was performed to evaluate the predictive power of baseline variables and viral sensitivity parameters for treatment outcome. Calculations including generation of receiver operating characteristic (ROC) curves were performed using SPSS version 10.0 (SPSS Inc., Chicago, IL, USA). A P-value <0.05 was considered to be significant.

Results

Clinical outcome

Table 1 shows baseline characteristics of the 46 consecutive patients who started therapy. There was neither any significant difference between responders and non-responders (irrespective of the response criteria applied) in either group nor between the three treatment groups with respect to baseline alanine aminotransferase (ALT), age, sex, weight, body mass index (BMI), fibrosis score and viral load (data not shown). In addition, for the whole group of 46 patients with genotype 4, none of these parameters were different compared with the historic genotype 1 sample (as described in Table 2 and in the Discussion).

In the PEG-IFN-α2a/RBV group, 2/33 patients with EVR dropped out (after 3 and 8 months of combination therapy, respectively). Treatment was terminated in a further patient with EVR because of severe exacerbation of atopic dermatitis after 4 months. Eight patients did not achieve an EVR. Five of these eight patients had a slow virological response of which only one achieved an SVR. The overall SVR rate was 67%.

HCV genotypes

Genotype 4 was first determined by line probe assay in all 46 patients. Genotyping and subtyping by NS5B sequencing was possible in 44 samples; no PCR amplification was achieved in two samples. Subtypes
determined by sequencing were 4a (n=35), 4b (n=4), 4c (n=3), 4d (n=1) and 41 (n=1) [28]. Correlations between subtypes determined by line probe assay and subtypes determined by sequencing are reported elsewhere [27]. Viral sensitivity parameters were not affected by subtype (data not shown).

IFN sensitivity and dose dependency
Baseline viral load (day -21) in the whole group was 5.53 (median; range: 4.21–6.19; n=46), and was not different between patients subsequently treated with PEG-IFN-α2a (n=33), standard IFN or PEG-IFN-α2b in combination with RBV. In addition, baseline viral load did not differ between virological responders or non-responders (irrespective of the response criterion applied). After the first decline induced by the single 10 MU IFN-α2b injection (on day -21), viral load returned to baseline within 1 week and on day -14 it was 5.41 (4.12–6.19; P=0.50 compared to day -21, Wilcoxon signed-ranks test).

The median 24 h log_{10} change after 10 MU IFN-α2b (1.15 [0.08–2.48]) was higher than after 5 MU IFN-α2b (0.81 [-0.12–2.22]; P<0.0001, Wilcoxon signed-ranks test). The median difference between the 10 MU and 5 MU 24 h log_{10} changes, as well as log_{10} changes on 5 MU IFN-α2b daily from days -14 to -7 and from days -14 to -1, are given in Table 2. Log_{10} change between days -13 and -7 was 0.33 (-0.38–2.02), which was lower (P=0.001, Wilcoxon signed ranks test) than the 24 h log_{10} change after the first 5 MU dose on day -14, reflecting the second slower phase of viral decline in analogy to genotype 1.

We checked for correlations between viral kinetic parameters and the baseline variables given in Table 1. Pearson correlation coefficients for BMI were -0.301 (P=0.042) for the 24 h log_{10} change after 10 MU IFN-α2b and -0.312 (P=0.035) for the 24 h log_{10} change after 5 MU IFN-α2b. In view of the need for adjustment of α-values because of multiple tests, these correlations should be considered as approaching significance only. There were definitely no correlations for baseline ALT, weight and age. In addition, the degree of liver fibrosis did not affect viral kinetics (Kruskall–Wallis and Mann–Whitney U tests; data not shown).

Prediction of virological response to PEG-IFN-α2a/ RBV combination therapy
As the three combination therapy regimens used may have different efficacy, we restricted the analysis to the largest group of 33 patients treated with PEG-IFN-α2a/RBV. The numbers of patients treated with either IFN/RBV (n=6) or PEG-IFN-α2a/RBV (n=7) were too small to allow a meaningful analysis of prediction.

Comparison of log_{10} changes between patients with or without an EVR to PEG-IFN-α2a/RBV therapy
Figure 1 shows the log_{10} changes in viral load 24 h after 10 and 5 MU IFN-α in the 32 patients in whom EVR to PEG-IFN-α2a/RBV therapy was assessed. Figure 2 shows the time course of viral load during the 14 days on daily 5 MU IFN-α. Statistics including comparison of the log_{10} changes between patients with and without an EVR are given in Table 3.

Analysis of predictive parameters by binary logistic regression
We performed a stepwise forward logistic regression analysis with age, sex, BMI, baseline viral load, baseline ALT, and 24 h log_{10} changes after 10 and 5 MU IFN-α2b as well as log_{10} changes after 1 and 2 weeks on 5 MU IFN-α2b daily as effect factors and EVR as dependent variable. The log_{10} change in viral load after 2 weeks on 5 MU IFN-α2b daily was the best predictive parameter. However, the 24 h log_{10} change after 10 MU IFN-α2b remained in the model as well (significance of change: P=0.015).

When analysing SVR as a dependent variable by using the same effect factors, the 24 h log_{10} change after 10 MU IFN-α2b was the best predictive parameter. BMI remained in the model as well, but it did not significantly aid the prediction (significance of change: P=0.998).

Table 2. Log_{10} changes in viral load in HCV genotype 4 patients (compared with the published study in genotype 1 [20])

<table>
<thead>
<tr>
<th></th>
<th>Genotype 4 [n=46]</th>
<th>Genotype 1 [n=28]</th>
<th>P-value †</th>
</tr>
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<tbody>
<tr>
<td>24 h after 10 MU IFN-α2b</td>
<td>1.15 [0.08–2.48]</td>
<td>0.65 [-0.04–1.94]</td>
<td>0.060</td>
</tr>
<tr>
<td>24 h after 5 MU IFN-α2b</td>
<td>0.81 [-0.12–2.22]</td>
<td>0.34 [-0.05–2.16]</td>
<td>0.068</td>
</tr>
<tr>
<td>Difference 10 versus 5 MU</td>
<td>0.26 [-0.20–0.91]</td>
<td>0.22 [-0.68–0.92]</td>
<td>0.46</td>
</tr>
<tr>
<td>1 week 5 MU IFN-α2b/day</td>
<td>1.06 [-0.31–3.51]</td>
<td>0.63 [-0.07–2.95]</td>
<td>0.10</td>
</tr>
<tr>
<td>2 week 5 MU IFN-α2b/day</td>
<td>1.92 [-0.13–3.70]</td>
<td>0.96 [-0.13–3.42]</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Data are given as median (range). *Taken from [20] for comparison (see discussion). †Mann–Whitney U test. HCV, hepatitis C virus; IFN, interferon; MU, megaunits.
Figure 1. Decline in viral load following a single dose of IFN-α2a

Log$_{10}$ changes in viral load 24 h after 10 megaunits (MU) interferon (IFN; open triangles) and 24 h after 5 MU IFN (closed circles) in the 32 patients on pegylated (PEG)-IFN-α2a/ribavirin (RBV) therapy who were assessed for early virological response (EVR). Twenty-two patients with and eight patients without EVR are shown. Patient 16 relapsed (RL), and patients 23 and 24 were lost during follow-up (LFU). Patient 25 without EVR, but with slow virological response, achieved a sustained virological response (SVR) to subsequent PEG-IFN-α2a/RBV therapy. The respective statistics are given in Table 3.

Figure 2. Viral kinetics on IFN monotherapy

(A) Viral kinetics during the 2 weeks on 5 megaunits (MU) interferon (IFN) daily in the 21 patients who achieved an early virological response (EVR) as well as a sustained virological response (SVR; full squares), the two patients with early virological response (EVR) who were lost for follow-up (closed diamonds, dashed lines) and the one patient with EVR who suffered a relapse (full triangle) after pegylated (PEG)-IFN-α2a/ribavirin (RBV) therapy. (B) Viral kinetics during the 2 weeks on 5 MU IFN daily in the seven patients who achieved neither an EVR nor an SVR (open squares) and the one patient without EVR, but with slow virological response, who became a sustained virological responder (open triangle) after 12 months of PEG-IFN-α2a/RBV therapy.
ROC analysis

Figure 3A shows ROC curves for prediction of EVR as a function of log$_{10}$ change in viral load after 2 weeks on 5 MU IFN-α$_2$b daily and 24 h log$_{10}$ change after 10 MU IFN-α$_2$b. The area under curve (AUC) was 0.97 for both variables, which was significantly different from 0.5 ($P < 0.001$, in both cases). The baseline viral load AUC was 0.40, which was not different from 0.5 ($P = 0.42$). A log$_{10}$ change within 2 weeks on 5 MU IFN-α$_2$b daily $> 0.64$ had a specificity of 100% and a sensitivity of 92%, a positive predictive value of 100% and a negative predictive value of 80% for the prediction of EVR. A 24 h log$_{10}$ change after 10 MU IFN-α$_2$b $> 0.72$ had a specificity of 100%, a sensitivity of 83%, a positive predictive value of 100% and a negative predictive value of 67% for prediction of the EVR.

Figure 3B shows the ROC curve for the prediction of SVR as function of the 24 h log$_{10}$ change after 10 MU IFN-α$_2$b. The AUC was 0.91, which again was significantly different from 0.5 ($P = 0.001$). The baseline viral load AUC was 0.31, which again was not different from 0.5 ($P = 0.17$). A 24 h log$_{10}$ change after 10 MU IFN-α$_2$b $> 0.52$ had a specificity of 88%, a sensitivity of 100%, a positive predictive value of 96% and a negative predictive value of 100% for prediction of SVR.

Discussion

Previously, we have shown that the 24 h response to a single dose of standard IFN is a useful predictor of treatment outcome in HCV patients [20]. In this study, we extend this observation to patients infected with HCV genotype 4 treated with PEG-IFN-α2a/RBV combination therapy. We show that there is a similar large spectrum of IFN sensitivity as seen in patients infected with HCV genotype 1, including the presence of IFN-resistant strains (Figure 1).

We used the same viral kinetic protocol as in our previous study on genotype 1 HCV [20], which serves mainly three purposes: to provide 24 h log$_{10}$ changes after two different doses for evaluation of their respective and possibly different predictive power; to study

<table>
<thead>
<tr>
<th>Log change in viral load (genotype 4), IU/ml</th>
<th>EVR (n=24)</th>
<th>No EVR (n=8)*</th>
<th>P-value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 h after 10 MU IFN-α2b</td>
<td>1.26 (0.45–2.48)</td>
<td>0.35 (0.08–0.68)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>24 h after 5 MU IFN-α2b</td>
<td>0.94 (-0.08–2.22)</td>
<td>0.20 (-0.12–0.37)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Difference 10 versus 5 MU</td>
<td>0.39 (-0.20–0.85)</td>
<td>0.15 (-0.16–0.62)</td>
<td>0.11</td>
</tr>
<tr>
<td>1 week 5 MU IFN-α2b/day</td>
<td>1.54 (-0.17–3.51)</td>
<td>0.23 (-0.31–0.76)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2 weeks 5 MU IFN-α2b/day</td>
<td>2.11 (0.23–3.70)</td>
<td>0.21 (-0.13–0.63)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Data are given as median (range). *Taken from [20] for comparison (see discussion). †Mann–Whitney U test. EVR, early virological response; IFN, interferon; MU, megaunits; RBV, ribavirin.

(A) Receiver operating characteristic (ROC) curves with early virological response (EVR) as state variable and log$_{10}$ change in viral load after 2 weeks on 5 megaunits (MU) interferon (IFN)-α2b daily (area under the curve [AUC]=0.97, $P<0.001$, thick line), 24 h log$_{10}$ change after 10 MU IFN-α2b (AUC=0.97, $P<0.001$, thick line) and baseline viral load (AUC=0.40, $P=0.42$, dotted line) as test variables. A log$_{10}$ change within 2 weeks on 5 MU IFN-α2b daily $> 0.64$ had 100% specificity and 92% sensitivity for prediction of EVR. A 24 h log$_{10}$ change after 10 MU IFN-α2b $> 0.72$ had 100% specificity and 83% sensitivity for prediction of EVR. (B) ROC curves with sustained virological response (SVR) as state variable and 24 h log$_{10}$ change after 10 MU IFN-α2b (AUC=0.91, $P=0.001$, thick line) and baseline viral load (AUC=0.31, $P=0.17$, dotted line) as test variables. A 24 h log$_{10}$ change after 10 MU IFN-α2b $> 0.52$ had 88% specificity and 100% sensitivity for prediction of SVR.
dose dependency prospectively in each patient and not merely by comparison of treatment arms [29]; and to study the anticipated biphasic decline in viral load over 14 days of daily standard IFN administration, which was done in the most important studies on viral kinetics in the past [29]. Furthermore, to enable a comparison of viral kinetics in genotype 4 to a historic genotype 1 sample, IFN-α2b was used as test dose irrespective of which IFN was given after the test phase. Application of IFN-α2a, which has slightly different efficacy, at least in vitro [30], would have hampered this comparison.

Observation of viral kinetics on IFN in HCV genotype 4 infection was the primary aim of our study. The combination therapy regimen was not predefined from the beginning of the study. Patients were enrolled consecutively and the first six patients received IFN-α2b/RBV, the following seven patients received PEG-IFN-α2b (12kDa)/RBV and all patients thereafter received PEG-IFN-α2a (40kDa)/RBV combination therapy, reflecting the availability of the different IFN preparations after licensing in Austria. There was no randomization between groups because comparison of the different treatments was not an aim of this study.

The decline in viral load during 2 weeks on daily standard IFN-α was biphasic as in genotype 1 [29,31]. In addition, there was an excellent correlation of very early kinetic parameters with outcome of subsequent combination therapy (Table 3). As this analysis would have been confounded by mixing up the three combination regimens with possibly different efficacy, we restricted it to the largest treatment group, that is, PEG-IFN-α2a plus 1–1.2g RBV for 48 weeks (n=33). The log_{10} changes 24 h after 10 MU IFN-α or after 2 weeks of 5 MU IFN-α daily were superior for prediction of EVR – on ROC analysis both parameters showed an AUC of 0.97. The cutoff for the maximal combined test efficiency (sensitivity plus specificity) was a 24 h log_{10} change after 10 MU IFN-α2b of greater than 0.72, which had 100% specificity and 83% sensitivity for prediction of EVR. These are the same numbers as reported previously for prediction of EVR by a 24 h log_{10} change after 9 MU IFN-α2a in genotype 1 HCV patients [23]. Surprisingly, for prediction of SVR, the log_{10} change 24 h after 10 MU IFN-α was the single best parameter by ROC and binary logistic regression analysis with an AUC of 0.91, which, again, is significantly different from 0.5 (P=0.001). A 24 h log_{10} change after 10 MU IFN-α2b >0.52 had 88% specificity and 100% sensitivity for prediction of SVR, which, again, is similar to previously reported numbers [26]. However, 100% specificity was not reached before a log_{10} change of 1.88. Baseline viral load, a classic parameter used for patient stratification, was weakly, but not significantly, predictive because of the small patient number (Figures 3A and B).

Thus, in genotype 4 patients a 24 h log_{10} change after a single high-dose standard IFN-α injection is as powerful as in genotype 1 patients for the prediction of treatment outcome on PEG-IFN-α2a/RBV combination therapy. Unfortunately, lack of a 24 h response using the cutoffs given is not sufficiently accurate to justify exclusion of patients from subsequent PEG-IFN-α2a/RBV therapy. Similar findings were obtained in genotype 1 patients [26]. There may be several reasons for this observation. Firstly, the biological activities of PEG-IFN-α2a and standard IFN differ due to variable pharmacokinetic and pharmacodynamic properties, such as volume of distribution and speeds of resorption and clearance. Due to the delayed pharmacokinetics of PEG-IFN-α2a, very early viral kinetics on this drug cannot predict outcome at all [22,32]. The slow resorption of PEG-IFN-α2a results in initial plasma levels (within the first 24 h) that are considerably lower than those 24 h after the administration of the sixth dose, when the drug has reached steady-state concentrations [33]. Secondly, the optimal dose of standard IFN for prediction of outcome of PEG-IFN-α/RBV therapy has not yet been determined and is probably much higher than doses used in this or in previous studies on genotype 1 [23,6]. Thirdly, the changes in viral load after standard IFN-α may be underestimated because of non-linearity of quantitative assays in higher titre ranges [27].

The assessment of very early IFN response may be of scientific importance for patient stratification in trials to assure homogeneous study groups with respect to IFN responsiveness [26], but has a limited role in clinical practice. Another way to predict successful treatment may be the rapidity of viral response as determined by the time of qualitative PCR seroconversion. In a recently reported Austrian trial, patients achieving a week 4 virological response (rapid virological response, RVR) were treated for 24 weeks with PEG-IFN-α2a/RBV only, and had treated per protocol SVR rates of 84 and 95% in genotype 1 and genotype 4 patients, respectively [34]. These response rates are similar as in another study from Egypt reporting 86% SVR in patients with RVR on PEG-IFN-α2b/RBV treated for 24 weeks and 76% SVR in patients with EVR treated for 36 weeks [17]. We could not study the predictive power of RVR because our patients received IFN monotherapy for 2 weeks prior to the start of combination therapy with PEG-IFN-α. A significant viral load decline on daily 5 MU IFN-α may have facilitated an RVR in some patients. By contrast, the observation that even some patients without any significant viral load decline after 2 weeks of daily standard IFN achieved an EVR can be interpreted in terms of a...
greater efficacy of PEG-IFN-α/RBV therapy at least after 4–5 weeks, when PEG-IFN-α reaches steady-state plasma levels. Therefore, we think that our assumption that EVR was not affected by standard IFN pretreatment is justified. Furthermore, the single 10 MU IFN-α2b dose is unlikely to affect any of the applied response parameters on combination therapy because viral load returned to baseline within 1 week [35].

By comparison with our previously published genotype 1 patients [20] with similar baseline parameters (Table 2), IFN responsiveness tended to be higher (P<0.05 for 2 weeks on standard IFN daily) in genotype 4 than in genotype 1 patients. In addition to genotype, genetically determined host factors may modulate IFN responsiveness and these should be taken into account when interpreting data from studies conducted in different areas. For example, African and Hispanic Americans respond far worse than Caucasians, even when matched for genotype [36]. HCV genotype 4 originating in Egypt is spreading to middle and southern European populations by intravenous drug abuse [37]. In a French study, there was a higher SVR rate (57%) in immigrants from Egypt compared with native French patients (40%) despite more severe histological liver lesions in the former group [38], although compliance problems in intravenous drug abusers may have contributed to this difference. Furthermore, immunological background may be modulated by concomitant parasitic infection in areas prevalent with genotype 4 HCV [39].

In conclusion, the initial decline in viral load is faster in patients with genotype 4 than in genotype 1. Determination of very early viral kinetics in HCV genotype 4 is useful to predict treatment outcome in PEG-IFN-α2a/RBV therapy and may help to select patients who will most likely benefit from this therapy. Despite the inaccuracy of negative prediction this may be of value in countries like Egypt, where access to therapy is limited by economic reasons. Furthermore, given the similar large variation of IFN responsiveness in genotype 4 compared with genotype 1, it is likely that individualization of treatment regimens in terms of IFN and RBV doses, as well as duration, will be of value to reduce costs and side effects on the one hand, and to result in better outcomes on the other hand. In light of limited healthcare resources and high HCV prevalence in those countries, further studies to promote this concept are urgently needed.

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Disclosure statement

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References

Early viral kinetics in HCV genotype 4 patients


