Efficacy of lead-in silibinin and subsequent triple therapy in difficult-to-treat HIV/hepatitis C virus-coinfected patients

Braun, D l; Rauch, A; Durisch, N; Eberhard, N; Anagnostopoulos, A; Ledergerber, B; Metzner, K J; Böni, J; Weber, R; Fehr, J

Abstract: Objectives: The efficacy of current hepatitis C virus (HCV) triple therapy, including a protease inhibitor, is limited in HIV/HCV-coinfected patients with advanced liver fibrosis and nonresponse to previous peginterferon-ribavirin. These patients have a low chance (only 30%) of achieving a sustained virological response (SVR) during triple therapy and cannot wait for next-generation anti-HCV drugs. In a pilot study, we investigated the efficacy of a lead-in therapy with silibinin before triple therapy in difficult-to-treat patients. Methods: Inclusion criteria were HIV/HCV coinfection with advanced liver fibrosis and documented failure of previous peginterferon-ribavirin treatment. Intervention was lead-in therapy with intravenous silibinin 20 mg/kg/day for 14 days. Subsequently, peginterferon-ribavirin combined with telaprevir was initiated for 12 weeks, followed by peginterferon-ribavirin dual therapy until week 48 after initiation of triple therapy. The outcome measurements were HCV RNA after silibinin lead-in, at weeks 2, 4 and 12 of triple therapy, and SVR at week 24 after the end of treatment. Results: We examined six HIV/HCV-coinfected patients (four infected with genotype 1a). All had fibrosis grade METAVIR F3 and were on fully suppressive antiretroviral therapy. Mean HCV RNA decline after silibinin therapy was 2.6 log10 IU/mL (range 2–3 log10 IU/mL). Five of the six patients were virologically suppressed at weeks 2 and 4, and all six at week 12 of triple therapy. One experienced a viral breakthrough thereafter. Four of five patients (80%) showed an SVR 24. One patient had an SVR 12 but has not yet reached week 24. Conclusions: A lead-in with silibinin before triple therapy is highly effective and increases the probability of HCV treatment success in difficult-to-treat HIV/HCV-coinfected patients with advanced liver fibrosis and previous failure of peginterferon-ribavirin.

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Objectives
The efficacy of current hepatitis C virus (HCV) triple therapy, including a protease inhibitor, is limited in HIV/HCV-coinfected patients with advanced liver fibrosis and nonresponse to previous peginterferon-ribavirin. These patients have a low chance (only 30%) of achieving a sustained virological response (SVR) during triple therapy and cannot wait for next-generation anti-HCV drugs. In a pilot study, we investigated the efficacy of a lead-in therapy with silibinin before triple therapy in difficult-to-treat patients.

Methods
Inclusion criteria were HIV/HCV coinfection with advanced liver fibrosis and documented failure of previous peginterferon-ribavirin treatment. Intervention was lead-in therapy with intravenous silibinin 20 mg/kg/day for 14 days. Subsequently, peginterferon-ribavirin combined with telaprevir was initiated for 12 weeks, followed by peginterferon-ribavirin dual therapy until week 48 after initiation of triple therapy. The outcome measurements were HCV RNA after silibinin lead-in, at weeks 2, 4 and 12 of triple therapy, and SVR at week 24 after the end of treatment.

Results
We examined six HIV/HCV-coinfected patients (four infected with genotype 1a). All had fibrosis grade METAVIR ≥F3 and were on fully suppressive antiretroviral therapy. Mean HCV RNA decline after silibinin therapy was 2.6 log10 IU/mL (range 2–3 log10 IU/mL). Five of the six patients were virologically suppressed at weeks 2 and 4, and all six at week 12 of triple therapy. One experienced a viral breakthrough thereafter. Four of five patients (80%) showed an SVR 24. One patient had an SVR 12 but has not yet reached week 24.

Conclusions
A lead-in with silibinin before triple therapy is highly effective and increases the probability of HCV treatment success in difficult-to-treat HIV/HCV-coinfected patients with advanced liver fibrosis and previous failure of peginterferon-ribavirin.

Keywords: advanced liver fibrosis, hepatitis C virus, HIV/hepatitis C virus coinfection, nonresponse, null response, silibinin.

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boceprevir were approved in Switzerland at the end of 2011 as the first direct-acting anti-HCV drugs (DAAs) in combination with pegylated interferon alpha-2a plus ribavirin (peginterferon-ribavirin) triple therapy (TT) for HCV-monoinfected and HCV/HIV-coinfected patients with genotype 1. However, despite the availability of TT, treating HCV infection remains challenging in difficult-to-treat patients, such as HIV/HCV-coinfected patients with advanced liver fibrosis and previous failure of peginterferon-ribavirin therapy. These patients are at the highest risk for HCV-related complications [2]. At the same time, they have the lowest chance (around 30%) of achieving a sustained virological response (SVR) with TT, and they are at the highest risk for on-treatment virological failure [3]. As these patients cannot wait until the next generation of anti-HCV drugs is available, new therapeutic options to eradicate HCV and to prevent further progression and complications of HCV infection are urgently needed [4].

A promising drug is silibinin, which targets different steps in the HCV lifecycle [5]. A pilot study showed that administration of 20 mg/kg intravenous silibinin significantly reduced HCV RNA (reduction 3.02 mean ± standard deviation 1.01 log10 IU/mL) in 20 HCV-monoinfected patients with previous null response to peginterferon-ribavirin therapy [6].

Encouraged by these findings, we investigated the use of intravenous silibinin followed by TT in HIV/HCV-coinfected patients with advanced liver fibrosis and previous failure of peginterferon-ribavirin therapy. We used intravenous silibinin as a lead-in therapy with the aim of rapidly lowering HCV RNA before TT initiation, and thus increasing the chance of treatment success. We report the efficacy and tolerability of silibinin in six difficult-to-treat coinfected patients as a proof of principle.

**Methods**

**Objectives**

The primary aim was to describe the efficacy and safety of a 14-day lead-in of intravenous silibinin in HIV/HCV-coinfected patients with advanced liver fibrosis who failed on previous treatment with peginterferon-ribavirin. Our secondary aim was to assess the viral response to silibinin and the following TT, during and at the end of treatment.

**Patient selection and previous responses to HCV therapy**

We treated six patients coinfected with HIV/HCV genotype 1 with a silibinin lead-in and subsequent TT. Patients were selected based on a documented history of previous failure of peginterferon-ribavirin, including null response (NR), partial response (paR), relapse or viral breakthrough, and documented advanced liver fibrosis. Virological responses were defined as published elsewhere [7]. Advanced liver fibrosis was defined as a METAVIR fibrosis score ≥ 3 in at least one documented liver biopsy since diagnosis of HCV infection.

**Investigational product and mode of treatment**

Intravenous silibinin is commercially available as Legalon® SIL (Rottapharm Madaus, Cologne, Germany). Intravenous silibinin is commercially available as Legalon® SIL is approved for treatment of chronically HCV-infected patients with nonresponse to standard therapy with peginterferon-ribavirin. In Switzerland, Legalon® SIL is approved for compassionate use for the same indication. All patients received lead-in therapy with intravenous silibinin at 20 mg/kg/day once a day for 14 days. After a 14-day intravenous silibinin lead-in treatment, patients started standard TT on day 15, including telaprevir and peginterferon-ribavirin. TT was continued for 12 weeks, followed by 36 weeks of peginterferon-ribavirin.

**Assessment of HCV RNA, drug resistance testing, silibinin safety and tolerability, and antiretroviral drug levels**

A safety laboratory assessment was obtained on days 1, 8, 12 and 15 of silibinin lead-in, and during subsequent TT and peginterferon-ribavirin therapy. Serum HCV RNA levels were determined using Roche COBAS TaqMan v2.0 (Rotkreuz, Switzerland) (sensitivity range < 15 IU/mL). HCV RNA was obtained 2 weeks before the first silibinin administration, on day 1 of silibinin lead-in, and also on days 8, 12 and 15 of silibinin lead-in (i.e. week 0 of TT). HCV viral load was monitored during TT at weeks 2, 4 and 12, and during peginterferon-ribavirin dual therapy at weeks 24 and 48 after initiation of TT. HCV drug resistance testing was performed by Janssen Diagnostics (Raritan, NJ, USA) using a population-based sequencing assay to determine HCV NS3/4A sequence. The antiretroviral drug levels were measured at days 1 and 15 by liquid chromatography–tandem mass spectrometry with automated online sample extraction. The tolerability of intravenous silibinin was determined by daily clinical assessment during the silibinin treatment period.

**Results**

**Patient characteristics**

The six patients had a median age of 49 years (range 38–56 years) (Table 1). HCV transmission was via injecting drug use (n = 4), via contaminated blood products (n = 1) and
unknown \( (n = 1) \). All patients were on fully suppressive antiretroviral therapy (ART) \( (\text{i.e.} < 20 \text{ HIV-1 RNA copies/mL plasma}) \) and had a median CD4 T-cell count of 574 cells/\( \mu \text{l} \) (range 175–686 cells/\( \mu \text{l} \)). Genotype testing revealed genotype 1a \( (n = 4) \), genotype 1b \( (n = 1) \) and genotype 1e \( (n = 1) \). All patients had advanced liver fibrosis with a METAVIR fibrosis score of \( \geq 3 \), and a fibroscan stiffness of \( \geq 14 \text{ kPa} \). Patients had experienced a previous NR \( (n = 3) \), a paR \( (n = 1) \), a viral breakthrough \( (n = 1) \) and a relapse \( (n = 1) \) (Fig. 1a). The ART regimens consisted of atazanavir/ritonavir (ATV/r), raltegravir (RGV), efavirenz (EFV), tenofovir (TDF), emtricitabine (FTC), abacavir (ABC) and lamivudine (3TC).

**HCV and HIV-1 RNA during silibinin lead-in and HCV triple therapy**

During the 14 days of intravenous silibinin therapy, four of the six patients experienced a \( 3 \log_{10} \text{ IU/mL HCV RNA decline} \), and two patients a \( 2 \log_{10} \text{ IU/mL HCV RNA decline} \); the mean HCV RNA decline was \( 2.6 \log_{10} \text{ IU/mL} \) (Fig. 1b). After the silibinin lead-in therapy and initiation of TT, five of the six patients reached an HCV RNA below the limit of detection at week 2 of TT, and all six patients did so at week 12 of TT (Fig. 1b). During the subsequent peginterferon-ribavirin therapy, patient B, with the genotype 1e, had a viral breakthrough at week 32 after initiation of TT. Drug resistance testing of the HCV NS3 protease region from the failing patient revealed the I132V mutation, which may be associated with resistance to telaprevir. Four of five patients (80%) showed an SVR 24. One patient has not yet reached week 24 after the end of treatment but had an SVR at week 12 after the end of treatment.

**Table 1  Baseline characteristics of six HIV/hepatitis C virus (HCV)-coinfected patients**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Sex</th>
<th>CD4 count (cells/( \mu \text{l} ))</th>
<th>ART</th>
<th>GT</th>
<th>METAVIR</th>
<th>Fibroscan (kPa)</th>
<th>Preceding peginterferon-ribavirin</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>49</td>
<td>m</td>
<td>396</td>
<td>ATV/r TDF</td>
<td>1a</td>
<td>F3</td>
<td>35.8</td>
<td>Null response</td>
</tr>
<tr>
<td>B</td>
<td>48</td>
<td>f</td>
<td>520</td>
<td>ATV/r TDF/FTC</td>
<td>1e</td>
<td>F3</td>
<td>19.8</td>
<td>Null response</td>
</tr>
<tr>
<td>C</td>
<td>50</td>
<td>m</td>
<td>627</td>
<td>ATV/r TDF</td>
<td>1a</td>
<td>F3</td>
<td>18.4</td>
<td>Viral breakthrough</td>
</tr>
<tr>
<td>D</td>
<td>47</td>
<td>m</td>
<td>686</td>
<td>RGV ABC/3TC</td>
<td>1b</td>
<td>F3</td>
<td>NA</td>
<td>Partial response</td>
</tr>
<tr>
<td>E</td>
<td>56</td>
<td>m</td>
<td>678</td>
<td>ATV/r 3TC</td>
<td>1a</td>
<td>F3</td>
<td>14.3</td>
<td>Relapse</td>
</tr>
<tr>
<td>F</td>
<td>38</td>
<td>m</td>
<td>175</td>
<td>RGV/EFV TDF/FTC</td>
<td>1a</td>
<td>NA</td>
<td>24.6</td>
<td>Null response</td>
</tr>
</tbody>
</table>

**Fig. 1  Historical treatment response compared to our pilot study.** (a) Previous treatment failure in six HIV/hepatitis C virus (HCV)-coinfected patients with pegylated interferon alpha-2a plus ribavirin dual therapy (PR). The dotted line indicates the failing patient shown in panel b. (b) HCV RNA viral load in the same six patients during lead-in therapy with intravenous silibinin for 14 days followed by initiation of triple therapy on day 15 (i.e. week 0) for 12 weeks, and peginterferon-ribavirin dual therapy for 36 weeks. The black arrows indicate the different treatment durations with PR in the six patients. bld, below the level of detection; EOTR, end of treatment response; SVR 24, sustained virological response 24 weeks after the end of treatment; PR, pegylated interferon alpha-2a plus ribavirin.
Adverse events associated with intravenous silibinin

Intravenous silibinin was well tolerated without any serious adverse events. The most common adverse event was a sensation of heat in three of six patients that receded spontaneously.

Antiretroviral drug levels

Concentrations of antiretroviral drugs (i.e. ATV, RGV and EFV) were not significantly influenced by silibinin lead-in therapy. All results for trough levels were within the standard reference values (ATV: 0.7–1.0 mg/L; reference value $0.86 \pm 0.84$; RGV: 0.07–0.78; reference value $0.04–1.00$; EVF: 2.8–3.4; reference value $1.7 \pm 1.0$) [8].

Discussion

In this pilot study, a silibinin lead-in followed by TT, including the protease inhibitor telaprevir, led to an SVR 24 in four of five (80%) difficult-to-treat HIV/HCV-coinfected patients with previous failure of peginterferon-ribavirin treatment. We observed a rapid HCV RNA decline during silibinin treatment, which decreased below the limit of detection after 2 weeks of TT in five of the six patients.

An SVR 24 rate of 80% is high given the current data on treatment success of TT in HCV treatment-experienced patients with advanced liver fibrosis. The REALIZE (telaprevir for retreatment of HCV-infection) trial reported an SVR of only 29% in 72 HCV-monoinfected patients with previous NR to peginterferon-ribavirin, retreated with telaprevir-based TT [9]. On-treatment virological failure in the REALIZE trial occurred in 18% of patients overall (i.e. in 52% of those with prior NR, in 19% of those with paR and in 1% of relapsers) [3]. The French early-access programme Agence Nationale de Recherche sur le SIDA (ANRS) C020-CUPIC enrolled 674 monoinfected cirrhotic patients with HCV genotype 1 and reported SVR 12 rates of 40% in the telaprevir group and 41% in the boceprevir group. In a subgroup analysis, patients with previous paR and NR had substantially lower treatment responses, with SVR rates at week 12 of 32% and 29% in the telaprevir group, and of 40% and 11% in the boceprevir group, respectively [10]. Recently, a European cohort study of HIV/HCV-coinfected patients reported real-life on-treatment data: in the ANRS CO13-HEPAVIH cohort/ESCMID European Study, 69% of 65 participants were previous nonresponders, 80% were infected with HCV genotype 1a, and the mean transient elastometry was 19.3 kPa [11]. This study population was similar to ours, but the virological response at week 48 was only 17% in the telaprevir group and 33% in the boceprevir group. The lower response rate in the telaprevir group was explained by the higher fraction of patients with the unfavourable genotype 1a compared with the boceprevir group. In summary, the treatment responses in our pilot study were substantially higher than those of similar patient groups reported in the literature. Of note, the SVR would also be high (75%) if the individual with a relapse after previous treatment with peginterferon-ribavirin were excluded.

We hypothesize that the high on-treatment and the high SVR rate in our pilot study is related to the lead-in therapy with silibinin. The $2–3 \log_{10} \text{IU/mL}$ viral RNA decline during the silibinin lead-in and thus the lower viral load when starting TT might have been key for subsequent treatment success [12,13]. A lower baseline viral load led to more rapid viral clearance and presumably prevented the development of resistance against telaprevir. In patients with prior peginterferon-ribavirin failure, the cornerstone of TT is the protease inhibitor, as peginterferon-ribavirin turned out to be ‘weak’ partners with increased risk for the emergence of HCV resistance variants and viral breakthrough [14]. That the ongoing viral replication is associated with the development of HCV resistance mutations [15] is also supported by the results of our analyses of the HCV NS3 protease region, which revealed the 1132V mutation in the woman with a viral breakthrough harbouring the genotype 1e. This patient had a slower HCV RNA decline after treatment initiation compared with the viral slopes of the other five patients. She had an ongoing low-level viraemia until virological suppression was achieved at week 12, and she subsequently had a viral breakthrough at week 32.

Although new HCV therapeutics were recently approved by the US Food and Drug Administration, or will be approved soon, we are convinced that silibinin will be of value for selected difficult-to-treat patients. First, information about treatment outcome with newer DAAs in cirrhotic HIV/HCV-coinfected patients with previous NR is limited. Of note, for sofosbuvir there are no data from phase 3 trials for treatment-experienced patients with genotype 1. Furthermore, phase 3 trials reported significantly lower SVR rates in therapy-experienced patients with prior NR, paR or viral breakthrough, compared with therapy-naive patients [16,17]. Secondly, interferon-free oral drug combinations might soon become standard of care, but, in many trials with HIV/HCV-coinfected patients, new DAAs, including simprevir, faldaprevir, sofosbuvir and daclatasvir, were still combined with peginterferon-ribavirin, especially for genotype 1 [18,19]. Thirdly, extremely high costs of DAAs may limit access to therapy, and from a global perspective the newer DAAs are not available in many countries. In contrast, silibinin is commercially available and would be accepted for compassionate use in most countries. The very recently published British HIV Association guideline 2013 recommends that, for patients with genotype 1 and a current clinical
need for treatment, the standard of care should be a TT with peginterferon-ribavirin combined with either telaprevir or boceprevir [20]. Thus, the add-on of silibinin to peginterferon-ribavirin in combination with first-generation protease inhibitors may be a valuable and affordable alternative for treating patients with previous NR or paR, or patients with advanced fibrosis, and may increase the probability of achieving an SVR.

This proof of principle study with a surprisingly high SVR rate of 80% has limitations. First, the actual contribution of the silibinin lead-in to the SVR rate is difficult to estimate, as our trial was not randomized and did not include a placebo group without silibinin. Secondly, the number of patients was small, and thus the SVR rate must be confirmed in a larger trial. However, the strength of our study is the real-life setting, including patients who urgently need new treatment strategies.

In conclusion, our pilot study demonstrates as a proof of principle that a 14-day lead-in with intravenous silibinin followed by TT is effective in difficult-to-treat HIV/HCV-coinfected patients. This translates into HCV treatment success in these patients with advanced liver fibrosis and previous failure of peginterferon-ribavirin.

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Conflicts of interest: AR received remuneration for patient recruitment and for sitting on the advisory board of Janssen Cilag. BL received fees from Gilead for consultancy and from Gilead and GSK for lectures. JB and JF received money from the Federal Committee for Sexual Health, Switzerland. JF is a member of the advisory board of Merck Sharp & Dome and Janssen and received unrestricted and travel grants from Gilead, Merck Sharp & Dome, Janssen, Bristol-Myers Squibb, Roche, VIIIV, Abbott, and Boehringer Ingelheim. KM received grants from Gilead Sciences, Roche Diagnostics and MSD and travel grants from Gilead Sciences, Roche, MSD, Abbott and mib Dienstleistung GmbH. RW received travel grants from Abbott, Boehringer Ingelheim, Bristol-Myers Squibb, Gilead Sciences, GlaxoSmithKline, Merck Sharp & Dome, Pfizer, Roche, TRB Chemedica and Tibotec.

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