

Treatment of Hepatitis C-Virus–Reinfection After Liver Transplant with Silibinin in Nonresponders to Pegylated Interferon-based Therapy

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Abstract

Objectives: Hepatitis C-virus–persistence after orthotopic liver transplant leads to reduced patient and graft survival compared to other indications. Current interferon-based antiviral therapy of hepatitis C-virus–infection posttransplant provides a sustained response rate of 30% to 40%. This study, performed in an hepatitis C-virus-reinfected liver transplant population, examines the antiviral effect of intravenously administered silibinin, recently reported to exhibit strong antiviral properties in the natural setting of hepatitis C-virus–related liver disease.

Patients and Methods: Four patients after orthotopic liver transplant with hepatitis C-virus–recurrence, previously having not responded to peg-interferon-ribavirin therapy, were treated with intravenous silibinin and additionally, after the 10th day, with standard interferon-based therapy. Aminotransferases and hepatitis C-virus–RNA were measured during treatment.

Results: All patients demonstrated normalization of liver enzymes and significant decline of hepatitis C-virus–RNA measured at day 10 (mean 2.8 logarithmic levels: 1.7, 2.3, 2.9, and 4.3) during silibinin monotherapy. One patient cleared hepatitis C-virus–RNA under silibinin monotherapy and

another patient eliminated hepatitis C virus under subsequent interferon-based therapy. No adverse effects were observed during silibinin application. **Conclusions:** Intravenous silibinin is an effective therapeutic approach for treating hepatitis C-virus–reinfection after liver transplant and should be evaluated further.

Key words: Graft hepatitis C, Recurrence of pretransplant disease, Milk thistle, Alternative antiviral therapy

Introduction

Hepatitis C virus infection is one of the most-serious liver diseases leading to the development of liver cirrhosis and hepatocellular carcinoma. Over 170 million people are infected worldwide.^{1,2} About 1.5% of the European population are virus carriers and have progressive loss of liver function when untreated or treated without success.³ For patients with end-stage hepatitis C-virus–induced liver disease, liver transplantation remains the treatment of choice.^{4,5} Owing to the hepatitis C-virus–persistence, liver graft is regularly reinfected, which is one of the most-important issues after orthotopic liver transplant considering the spectrum of graft diseases. The recurrence of hepatitis C-virus–infection is usually more progressive compared with the natural course of the disease in nontransplanted patients.^{4,6,7} Most patients show either biochemical or histologic signs of inflammation, whereas 30% of all graft recipients develop graft cirrhosis within 5 years after transplant leading to impaired survival.^{4,5,7,8} Current antiviral therapy of C-virus–infection consists of applying pegylated interferon α -2a (IFN) and ribavirin (RBV) providing a sustained virologic response in 40% to 50% of all treated cases with

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hepatitis C virus genotype 1 and in 80% with genotypes 2 or 3.⁹⁻¹¹ After surgery, success of antiviral therapy is significantly lower, and a maximum of 30% to 40% of all patients achieve sustained virologic response.¹²⁻¹⁴ Therefore, a reasonable alternative therapy must complement standard therapeutic approach and prevent rapid development of graft fibrosis.

Silibinin experiences its renaissance in the treatment of chronic liver diseases although it has been known for years as a hepatoprotective herbal remedy and used by patients with chronic liver disease of various causes.^{13, 15-18} Next to the 3 other flavonolignan isomers (isosilybin, syldianin and silychristin), it is the most pharmacologically active component of *silybum marianum* and has been shown to improve biochemical markers of liver function and symptoms in a range of conditions including acute and chronic viral hepatitis, alcoholic liver disease and drug-related liver injury.^{11, 17, 19, 20} Silibinin exerts antifibrotic and anti-inflammatory effects by suppressing important fibrogenic cytokines such as transforming growth factor- β 1 (TGF- β 1).^{19, 21} Silibinin is a potent inhibitor of human hepatic stellate cells in vitro, which are the major source of the extracellular matrix and subsequently of hepatic scar tissue formation.²²⁻²⁵ As a potent free radical scavenger, this substance has been shown to reduce the initiation of pathogenetically important lipid peroxidation and to exert a direct antiviral effect on the hepatitis C-virus-replicon system by inhibiting RNA-dependant hepatitis C-virus-polymerase.^{23, 26} Recent reports demonstrate significant antiviral properties of silibinin in former nonresponders to standard antiviral therapy in the natural course of the hepatitis C-virus-associated liver disease when administered as high-dose intravenous monotherapy followed by repetitive IFN-RBV-application.^{11, 27} No data on antiviral silibinin efficacy in IFN-nonresponding graft recipients are available. This report focuses on the first experiences with silibinin in treating hepatitis C-virus-reinfection after liver transplantation.

Patients and Methods

Limited IFN-RBV therapy success in the posttransplant setting forced us to treat 4 nonresponders with intravenous silibinin and repetitive IFN-RBV-applications. Sustained virologic

response was defined as hepatitis C-virus-negativity at least 6 months after therapy completion, while detectable hepatitis C-viral-load after 6 months was considered an overall therapy failure. Aspartate aminotransferase, alanine aminotransferase (AST, ALT [1 U/L = 0.016 μ mol/L]) and hepatitis C-virus-RNA (Cobas AmpliPrep/ TaqMan/Roche/lower threshold of detection 15 IU/mL) were measured during treatment and follow-up. The protocol was approved by our local institutional ethics committee conforming to the ethical guidelines of the 1975 Helsinki Declaration. Written, informed consent was obtained from all subjects.

As nonresponder to IFN-based antiviral treatment, patient 1 underwent orthotopic liver transplantation for the end-stage hepatitis C-virus-induced liver cirrhosis, quickly achieving a stable graft function. Thirty-two months later, graft hepatitis was confirmed biochemically (AST = 256 U/L, ALT = 294 U/L), virologically (hepatitis C viral load = 17 700 000 IU/mL) and histologically as moderate inflammation (grade 2) in a moderate graft fibrosis (stage 2). IFN-RBV therapy was tried twice after orthotopic liver transplantation, but was aborted owing to an absent therapy response and considerable hematologic adverse events. Tacrolimus was used as immunosuppression at a dosage of 2 mg per day.

Patient 2 underwent orthotopic liver transplantation for hepatocellular carcinoma in hepatitis C-virus-induced liver cirrhosis without previous antiviral therapy. Stabilization of graft function was achieved quickly. The patient had to be readmitted to the hospital owing to severe hepatitis C-virus-reinfection with progressive loss of liver function. Pegylated interferon α -2a and ribavirin therapy was initiated but was aborted owing to nonresponse, adverse effects, and continuing graft function impairment. Histologic and laboratory examinations, performed merely 1 year after orthotopic liver transplant, revealed hepatitis C-virus-induced inflammation grade 3, fibrosis stage 4, viral load of 268 000 IU/mL, and slightly elevated aminotransferases (AST = 91 U/L, ALT = 52 U/L). Owing to an imminent graft failure, the patient was considered for retransplant.

Patient 3 had been treated with interferon and ribavirin without success before orthotopic liver transplantation for hepatitis C-virus-induced end-stage liver disease. After successful transplant

biochemically (AST = 243 U/L, ALT = 495 U/L) and histologically confirmed hepatitis C-virus-graft inflammation (grade 2) and fibrosis (stage 2) indicated the necessity of antiviral treatment, hepatitis C-viral-load at the beginning of therapy with intravenous silibinin was 20 700 000 IU/mL. Immunosuppressive therapy consisted of 2 mg of tacrolimus and 2 g of mycophenolate mofetil twice a day.

Patient 4 successfully underwent an orthotopic liver transplantation for hepatocellular carcinoma in hepatitis C-virus-induced cirrhosis after unsuccessful IFN-based antiviral treatment. Owing to hepatitis C-virus-induced graft hepatitis, confirmed by histologic (inflammation grade 2, fibrosis stage 2), laboratory (AST = 168 U/L, ALT = 136 U/L), and virologic (hepatitis C viral load of 25 000 000 IU/mL) analyses, the necessity of antiviral therapy seemed to be indispensable. Immunosuppression consisted of 2 mg tacrolimus and 2 g mycophenolate mofetil per day.

All patients were nonresponders to previous peg-interferon alpha 2a and ribavirin antiviral treatment before and after orthotopic liver transplant. Therapy failure was defined as *viral persistence 6 months after therapy completion* including primary nonresponse therapy failure and relapse.

Owing to significant hepatitis C-virus-recurrence after primarily successful liver transplant, all patients were hospitalized for initiating an alternative mode of antiviral treatment comprising intravenous silibinin and subsequent standard IFN-RBV application. Silibinin was administered in 4 infusions (20 mg/kg) for 14 days, before IFN-RBV start on the 10th day at recommended dosages. Quantitative assessment of viral load and serum concentration of parenchymal enzymes was performed to monitor the antiviral effect of silibinin (Table 1; Figures 1, 2). Immunosuppression was not modified before, during, or after therapy. Demographic data and pretreatment characteristics are displayed in Table 2.

Results

Four patients with graft hepatitis C-virus-reinfection after orthotopic liver transplantation were treated with intravenous silibinin for 14 days and IFN-RBV complementation at day 10. Standard therapy could not be performed in patient 2 owing to expected adverse effects of IFN-RBV,

Table 1. Demographic data.

	Patient 1	Patient 2	Patient 3	Patient 4
OLT Indication	HCV-Cirrhosis	HCC/HCV-Cirrhosis	HCV-Cirrhosis	HCC/HCV-Cirrhosis
Months after OLT	32	8	6	6
Sex	Female	Male	Male	Male
Donor age (y)	33	56	30	59
Recipient age (y)	46	58	44	66
Previous treatment (n)	3	2	1	2
Immunosuppression	Tac	Tac/MMF	Tac/MMF	Tac/MMF
HCV-Genotype	1b	1a	1b	1b
Grade of inflammation	2	3	2	2
Stage of fibrosis	2	4	2	2
Viral load [IU/mL]	17 700 000	268 000	20 700 000	25 000 000

Demographic data and pretreatment condition.

Histologic grade of inflammation and stage of fibrosis according to Desmet and Scheuer using a scale of 0 to 3 for the inflammatory activity criteria (0: normal; 1: mild; 2: moderate; 3: severe) and 0 to 4 for fibrosis staging (0: absent, 1: mild without septa, 2: moderate with few septa, 3: numerous septa without cirrhosis and 4: cirrhosis).

Abbreviations: HCV, hepatitis C virus; MMF, mycophenolate mofetil; OLT, orthotopic liver transplant; Tac, tacrolimus

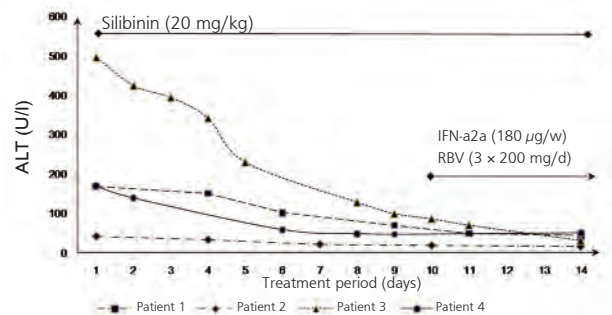


Figure 1. Decline of alanine aminotransferase during silibinin treatment.

as previously seen even at lower dosage regimen (IFN 135 µg/w and RBV 200 mg/d).

While silibinin was administered intravenously, aminotransferases of all 4 patients normalized within 10 days (Figure 1, Table 2). Rapid and most-prominent decline of hepatitis C-virus-RNA was observed in all 4 cases during the silibinin treatment. A 2.8 log drop in viremia was achieved in average, ranging from 1.7 to 4.3 (Figure 2). Histologic analysis of graft damage was performed before treatment only.

Two of 4 patients (1 and 2) became hepatitis C-virus-negative. Patient 1 lowered the hepatitis C-viral-load by 1.7 log steps during silibinin infusion, and eventually eliminated hepatitis C virus under IFN-RBV standard therapy 2 months later. Fibrosis scores differed substantially (stage 2 in patient 1 and stage 4 in patient 2).

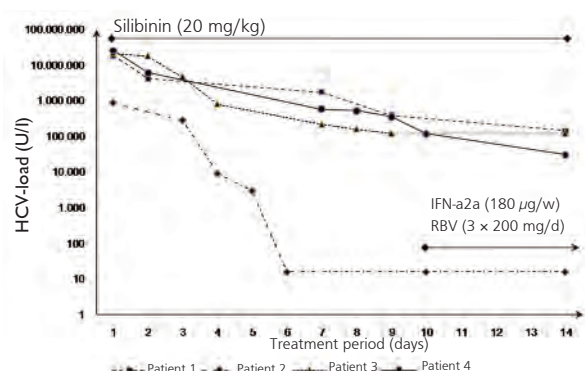
With initially low hepatitis C-viral-load (268 000 IU/mL), patient 2 eliminated the virus

Table 2. Virologic and laboratory effect of intravenous silibinin administration.

			Patient 1	Patient 2	Patient 3	Patient 4
HCV load (IU/mL)	AVT	day 1	17 700 000	268 000	20 700 000	25 000 000
		day 10	377 000	undetectable	115 000	29 000
	After AVT	week 12	undetectable	undetectable	11 400 000	3 800 000
		week 24	undetectable	undetectable	14 460 000	1 190 000
Log drop		day 1-10	1.7	4.3	2.3	2.9
AST / ALT (U/L)	AVT	day 1	256/294	91/52	243/495	168/136
		day 10	38/41	23/13	21/29	44/37
	After AVT	week 12	24/14	26/8	198/302	94/37
		week 24	28/15	26/9	176 /298	91/35

Virologic and laboratory effect of intravenous silibinin administration in treated patients displaying viral load, logarithmic difference (decadic) and aminotransferases (ALT / AST) during the therapy at day 1 and 10. Viral load and aminotransferases 12 and 24 weeks after treatment completion.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; AVT, antiviral treatment; HCV, hepatitis C virus

**Figure 2.** Decline of HCV-load during silibinin treatment.

during the first week of silibinin treatment alone and achieved sustained virologic response even without IFN-RBV standard therapy. Patients 1 and 2 were followed up for more than 1 year in good health demonstrating clear improvement of graft function monitored by routine laboratory analyses.

Initially demonstrating viral reduction during silibinin treatment, patients 3 and 4 virologically broke through, despite complementary IFN-RBV. As soon as silibinin pretreatment was finished, virologic and biochemical signs of graft hepatitis returned. After the initial viremia decline of 2.3 log levels under silibinin monotherapy (115 000 IU/mL), patient 3 increased the viral load up to 1 980 000 IU/mL during IFN-RBV therapy.

Patient 4 demonstrated a significant viral decline of 2.9 log steps (from 25 000 000 IU/mL to 30 000 IU/mL) during silibinin administration, principally suggesting a good therapy response. Under subsequent IFN-RBV treatment, the viral load increased up to 3 300 000 IU/mL.

Silibinin was well tolerated by all 4 patients without any unfavorable effects. No adverse events occurred, either during the intravenous silibinin

administration or subsequent observation. The dynamics of hepatitis C-viral-RNA and ALT are depicted in Figures 1 and 2.

Discussion

The antiviral effect of high-dose intravenous silibinin administration in combination with peg-interferon alpha 2a and ribavirin standard therapy was evaluated in 4 posttransplant patients with significant graft hepatitis. To the best of our knowledge, these findings, despite the small patient population, are the first to be reported on the antiviral efficacy of intravenously administered silibinin in a reinfected liver transplant population, nonresponsive to previous IFN-based therapy. The remarkable viral reduction (2.8 log levels) accompanied by the normalization of aminotransferases (Figures 1 and 2) observed during intravenous silibinin administration leads to the assumption that silibinin might exhibit a direct antiviral effect. Our results are in accord with observations made in a cohort of nontransplant patients with hepatitis C-virus-related liver disease.^{11, 27}

Evidence of antiviral efficacy of silibinin in patients with hepatitis C-virus-related liver damage is limited owing to a paucity of representative clinical trials despite its popularity among patients with various chronic liver diseases.^{13, 15, 16} Even less is known about therapeutic effect of silibinin in hepatitis C-virus-infected patients after liver transplant. Hawke and associates treated 24 cirrhotic chronic hepatitis C patients, who previously had failed interferon-based treatment, with orally administered silibinin up to 2.1 g/d exceeding customary dosage. The authors concluded that the

plasma steady-state concentration of the major flavonolignans (silybin A/B) depended upon the dosage exposure in a nonlinear manner. Low bioavailability of orally administered silibinin has been explained by a strong first pass effect. No significant effect on either aminotransferases or hepatitis C viral-load could be observed in the trial.¹⁸ Similarly, Gordon and associates tested orally administered silybum marianum (600 and 1200 mg for 12 weeks) in a double-blind placebo controlled setting of 20 chronic hepatitis-C patients demonstrating no significant adverse events during the treatment and no virologic effects, based on serum aminotransferases and hepatitis C viral load.¹³ Contrary, Loguercio and associates demonstrated that oral application of silibinin-vitamin-E-phospholipid complex significantly correlated with indices of fibrosis, plasma levels of fibrogenic TGF- β 1 and inflammatory cytokines (eg, tumor necrosis factor- β) in a cohort predominantly consisting of patients with nonalcoholic fatty liver disease and 26 chronic hepatitis-C patients using twice the amount than usual. The authors concluded that silibinin might have a hepatoprotective and antifibrogenic effect when administered at higher dosages with vitamin-E-phospholipid complex suggesting a dose-dependent therapeutic effect.¹⁹ Considering the bioavailability of oral silibinin (2% to 3%), it is likely not to reach therapeutic plasma concentration.²⁸ Owing to unfavorable pharmacokinetics properties, silibinin might erroneously be disregarded as potentially helpful antiviral agent despite implicit historical and recent hints explaining its current limited use. Therefore, it remains a matter of speculation because sufficient clinical trials have not been performed.

Intravenous application of silibinin seems to have more apparent antiviral effects and parenchymal protection, as demonstrated in our hepatitis C-virus-reinfected transplant patients and in the natural course of the hepatitis C-virus-induced liver disease.^{11, 27} Ferenci and associates provided most-reliable results in a trial with 36 hepatitis C-virus-positive patients treated with intravenous silibinin and IFN-RBV, convincingly suggesting a dose- and treatment-duration-dependent antiviral effect of silibinin. An overall hepatitis C-viral-load drop of 4.85 logarithmic levels was achieved, whereas 7 patients eliminated hepatitis C virus at week 12 after silibinin pretreatment (15-20 mg/kg/d) for 2 weeks

and subsequent interferon-based therapy. Similar therapy results were observed by Biermer and associates in a patient with hepatocellular carcinoma due to hepatitis C virus infection who had previously not responded to IFN-RBV therapy. After intravenous application of silibinin (20 mg/kg silibinin for 1 week), a 2.4 log drop of viremia was documented.²⁷

In our study, intravenous silibinin was analogously applied at 20 mg/kg with inspiring results leading to sustained virologic response in 2 patients. Normalization of hepatic inflammation markers (AST/ALT) and exponential decline of viral load during treatment strongly support the results demonstrated by Ferenci and Biermer, as the most-prominent viral load decline in all 4 patients during silibinin application. The question of adequate treatment duration arises. Regarding the hepatitis C-virus-decline (Figure 2), the antiviral effect seems to depend upon the interindividual capability of viral elimination, initial viral load, and silibinin treatment duration. A matter of speculation is the duration of silibinin treatment exceeding the proposed 2 weeks.

Dosage and order of silibinin application seem to be relevant issues. Peg-interferon alpha 2a and ribavirin therapy in chronic hepatitis C-virus-infection have been reported to synergistically impair the mitochondrial function triggering formation of reactive oxygen species, free radicals, and the initiation of lipid peroxidation.^{29, 30} To avoid unfavorable oxidative stress (which is considered to be one of the most-important pathogenetic columns in tissue damage and fibrogenesis), it might be reasonable to use the antioxidative properties of silibinin as radical scavenger before application of IFN-RBV. The silibinin dose tried in our patients corresponding to the pharmacologic recommendations of 20 mg/kg (MADAUS GmbH, 51101 Koeln, Germany, drug registration number 4178.00.00) showed no adverse effects. Administering higher quantities of intravenous silibinin should be discussed, especially regarding dosage-related antiviral efficacy and near-perfect adverse effect profile at the investigated dosage.¹¹ As a safe and potent antiviral substance, intravenous silibinin may be considered at least as a supplementation to current antiviral therapy guidelines, regarding IFN-independent reduction of viral load and normalization of laboratory markers demonstrated in our patients.

While performing a strenuous, expensive, and frequently futile effort of virus elimination, 60% to 70% of transplants with hepatitis C-virus-graft hepatitis are still treated without sustained virologic response leaving overall poor posttransplant antiviral treatment results. The major advantage of peg-interferon alpha 2a and ribavirin-therapy is in the antiviral potency of both substances in combination, reaching sustained virologic response rates of 30% under transplant condition, whereas their antiviral potency itemized is rather low.^{31, 32}

Thus, any kind of antiviral supplementation to current therapy regimen should be welcome in the age of donor organ shortage to strengthen current antiviral therapy regimen and to avoid graft loss with subsequent retransplant. Randomized, placebo-controlled clinical trials with a representative number of graft recipients with hepatitis C-virus-reinfection are needed to answer definitely the question of the efficacy of intravenous silybium marianum solution and its potential role in antiviral treatment.

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