

Silymarin use and liver disease progression in the Hepatitis C Antiviral Long-Term Treatment against Cirrhosis trial

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Publication data

Submitted 12 July 2010
First decision 31 July 2010
Resubmitted 7 October 2010
Accepted 11 October 2010
EV Pub Online 2 November 2010

SUMMARY

Background

Silymarin is the most commonly used herbal product for chronic liver disease; yet, whether silymarin protects against liver disease progression remains unclear.

Aim

To assess the effects of silymarin use on subsequent liver disease progression in 1049 patients of the Hepatitis C Antiviral Long-Term Treatment against Cirrhosis (HALT-C) trial who had advanced fibrosis or cirrhosis and had failed prior peginterferon plus ribavirin treatment.

Methods

Patients recorded their use of silymarin at baseline and were followed up for liver disease progression (two point increase in Ishak fibrosis score across baseline, year 1.5, and year 3.5 biopsies) and over 8.65 years for clinical outcomes.

Results

At baseline, 34% of patients had used silymarin, half of whom were current users. Use of silymarin was associated ($P < 0.05$) with male gender; oesophageal varices; higher ALT and albumin; and lower AST/ALT ratio, among other features. Baseline users had less hepatic collagen content on study biopsies and had less histological progression (HR: 0.57, 95% CI: 0.33–1.00; P -trend for longer duration of use=0.026). No effect was seen for clinical outcomes.

Conclusions

Silymarin use among patients with advanced hepatitis C-related liver disease is associated with reduced progression from fibrosis to cirrhosis, but has no impact on clinical outcomes (Clinicaltrials.gov #NCT00006164).

Aliment Pharmacol Ther 2011; 33: 127–137

INTRODUCTION

An estimated 130–170 million individuals are chronically infected with hepatitis C virus worldwide.¹ Pegylated interferon and ribavirin treatment results in an approximate sustained virological response rate of 55%.^{2, 3} However, individuals who fail to respond or who are unable to tolerate treatment have few additional options. As such, many patients have turned to complementary and alternative medications (CAM) instead of, or in addition to, standard therapy.^{4, 5}

An extract of the milk thistle plant, silymarin (*Silybum marianum*), has been used to treat chronic liver disease since the time of the ancient Greeks.⁶ Silymarin is the most commonly used herbal product by individuals with chronic liver disease, and a recent publication from the Hepatitis C Antiviral Long-Term Treatment against Cirrhosis (HALT-C) trial indicated that nearly one-third of patients in the trial were former or current users.⁷ Although the exact chemical composition of preparations varies, silymarin consists of a mixture of flavonoids termed flavonolignans.⁸ Results from laboratory, animal and clinical studies suggest that silymarin may have anti-inflammatory,^{9–11} anti-viral,^{11–14} antioxidant,^{10, 15} and antifibrotic effects in the liver.^{10, 16, 17} However, clinical efficacy, particularly in the context of chronic hepatitis C, remains unproven, and results from most previous studies, including randomised trials, are inconsistent.^{5, 6}

As an *a priori* hypothesis, information on baseline silymarin use was collected as part of the HALT-C trial. In the current report, we examined the association of baseline silymarin use with subsequent liver disease progression in 1049 patients with advanced chronic hepatitis C.

PATIENTS AND METHODS

The HALT-C trial was designed to evaluate the efficacy of long-term treatment with low-dose peginterferon alpha-2a for patients with hepatitis C-related bridging fibrosis and cirrhosis who had failed standard of care peginterferon plus ribavirin therapy.¹⁸ Patients were recruited from ten US medical centres and met the following criteria: detectable HCV RNA; nonresponse to prior peginterferon/ribavirin therapy; hepatic bridging fibrosis or cirrhosis on liver biopsy (Ishak fibrosis stage ≥ 2); and the absence of defined exclusion criteria (such as liver disease other than hepatitis C or history of hepatic decompensation or HCC).

Study design

A detailed description of the design of the HALT-C Trial is published.¹⁸ Patients whose previous failed treatment

had not included peginterferon plus ribavirin were treated with this therapeutic combination as part of the 'lead-in' phase of the trial. If patients had detectable HCV RNA at 20 weeks of treatment, they were considered nonresponders and included in the randomised phase of the trial. Responders, who had undetectable HCV RNA at 20 weeks of treatment, received peginterferon plus ribavirin treatment for 48 weeks. Patients experiencing breakthrough, defined by detectable HCV RNA between 20 and 48 weeks of treatment, and patients who relapsed after completion of 48 weeks of therapy, could also enrol in the randomised phase of the trial. Finally, patients who upon recruitment had already failed peginterferon plus ribavirin therapy were immediately entered into the randomised phase of the trial (express patients).

During the randomised phase of the trial, patients were randomised to peginterferon alpha-2a 90 mcg weekly or no treatment. Liver biopsies were repeated 1.5 and 3.5 years after randomisation. A panel of twelve hepatic pathologists reviewed all biopsies and scored inflammation (0–18) and fibrosis (0–6) using the Ishak scoring system.¹⁹ As peginterferon therapy did not affect clinical outcome or histological progression,¹⁸ treated and untreated participant data from the randomised phase of the trial were combined.

Assessment of silymarin use

At baseline, trained study coordinators obtained patient medication use by way of an in-person interview. In addition to assessing prescription and nonprescription drugs, interviewers assessed CAM (herbal medications, dietary supplements and botanical products). Patients who used CAM at least once a week for 1 month or longer in their lives were defined as users. Duration of use was also recorded. To facilitate recall, patients were shown a card indicating 37 examples of herbal products in alphabetical order. Silymarin was one of these products. Patients also had the opportunity to indicate use of herbal products not listed on the card. Every 3 months after baseline, and as often as every 2 weeks during the lead-in phase, patients were asked whether they had stopped using any herbal products since their last visit or whether they were taking any new herbal products. Of 1050 randomised participants, we excluded one patient who lacked a medication record. We considered current users to be those using silymarin on the day of study randomisation. Former users could have used silymarin at any time prior to randomisation, including the lead-in phase. Duration of silymarin use included all

months of use up to the day of randomisation. To analyse duration, we created a three level variable which included never users as the reference category. Months of use were then split at the median (16.6 months).

Morphometric image analysis of hepatic collagen content

Collagen in liver biopsy sections was stained with Sirius red and the degree of staining, which is proportional to the collagen content, was assessed using Image PRO PLUS 6.0 imaging software (Media Cybernetics, Silver Spring, MD, USA) as previously described.^{20, 21} Sirius red values are expressed in arbitrary units, but reflect collagen content on a continuous scale. Analyses of collagen content were restricted to unfragmented biopsies with more than 10 mm² of liver tissue in the section to avoid artefact and to reduce sampling variability. In total, 558 patients had morphometric image analysis performed on baseline biopsies, 550 patients had year 1.5 biopsies assessed by morphometry and 409 patients had year 3.5 biopsies analysed in this way. Of these patients, 183 had all three biopsies read.

Assessment of fibrosis and clinical outcomes

Patients were seen every 3 months in the randomised phase, at which point, complete blood counts, a liver chemistry panel and alpha-fetoprotein were tested at each clinical site. Patients also had at least one abdominal ultrasound examination every 12 months. Clinical outcomes included ascites, Child-Turcotte-Pugh score of ≥ 7 ²² on two consecutive study visits, liver disease-related death, hepatic encephalopathy, hepatocellular carcinoma (HCC), spontaneous bacterial peritonitis, or variceal haemorrhage. Outcome reports were reviewed by an Outcomes Review Panel consisting of three investigators from the participating clinical centres. Participants with bridging fibrosis at baseline and a ≥ 2 point increase in Ishak fibrosis score (TPI) on either of the follow-up biopsies were considered to have fibrosis progression. Results are presented separately for clinical outcomes and for a two point increase in Ishak fibrosis score as well as for the two endpoints combined. HCC was diagnosed via ultrasound, AFP and histological confirmation as previously described.²³

All details of this study were approved by the local Institutional Review Board at each participating institution, and all participants gave written informed consent.

Statistical analyses

We performed analyses using SAS release 9.1 (SAS Institute, Cary, NC, USA). All tests were two-sided and an

alpha level of <0.05 was considered statistically significant.

We tabulated baseline demographic, behavioural and clinical factors by categories of silymarin use (never, former, and current). Statistically significant variation across categories of silymarin use was assessed for categorical variables by the Mantel-Haenszel test for trend and for continuous variables with the Jonckheere-Terpstra test of trend.

Relative risks and 95% confidence intervals for the association of silymarin use with disease progression were calculated by use of Cox proportional hazards regression.²⁴ Person-time was calculated from baseline to first outcome, end of study, or date of patient withdrawal. For clinical outcomes and the combined endpoint, follow-up time was up to 8.65 years. For TPI, follow-up was until first (1.5 years) or second biopsy (3.5 years). For clinical outcomes, Kaplan-Meier curves were generated for never, former and current silymarin users and were compared with the log-rank test.

Linear trend tests across increasing categories of silymarin use were performed by creating an ordinal variable for each category and entering the term as a continuous variable into the regression model. We tested the proportional hazards assumption by modelling interaction terms of time with trend variables for silymarin use. No significant deviations were found for silymarin use with either TPI or clinical outcomes; however, a significant deviation was found for HCC ($P = 0.011$).

Relative risks for liver disease progression were estimated from crude models and two different multivariate adjusted models. The first multivariate model included continuous age, lifetime alcohol use and coffee intake along with categorical variables for education (high school or less, some post high school, completed college), race/ethnicity (Caucasian, African American, Hispanic and other), gender, diabetes and ever use of other herbal products besides silymarin, such as green tea, garlic, ginseng, or echinacea. A second multivariate model was additionally adjusted for continuous mental and physical short-form (SF)-36 summary quality of life scores,²⁵ and serological predictors of liver disease progression²⁶ – AST/ALT ratio, albumin, platelets, bilirubin, as well as categorical variables for cirrhosis status at baseline and presence or absence of oesophageal varices. For analyses of silymarin use during the trial, we updated silymarin use at the time of each follow-up biopsy. As for the main analyses, patients with a TPI at biopsy one (year 1.5) were censored at this time.

We assessed possible effect modification (interaction) by randomisation group, cirrhosis at baseline, median mental, and physical SF-36 quality of life scores and gender using stratification. Risk estimates did not vary by stratification group ($P > 0.23$ for all). Results stratified by cirrhosis at baseline are presented in the results section.

Finally, we analysed changes in morphometric collagen content across study biopsies using repeated analysis of variance, assuming an autoregressive covariance structure with the PROC MIXED function of SAS 9.1. Again, analyses were restricted to patients who had not had an outcome prior to a particular scheduled study biopsy. Time (baseline, biopsy one, and biopsy two) and silymarin use (former and current) were included in the model as fixed effects. Adjustment for age and gender did not alter risk estimates and so were not included in the final models. Possible differences between the collagen content of biopsies taken from former or current silymarin users were compared with the collagen content of biopsies taken from never silymarin users by the Mann–Whitney test.

RESULTS

At baseline, 17% (178/1049) of patients were former users of silymarin and 16% (170/1049) of patients were current users compared to 67% (701/1049) who reported never using silymarin (Table 1). The median duration of use for current users up to study entry was 35 months, whereas the median duration of use for former users was 6 months. Baseline silymarin use was associated with Caucasian race, completing college, male gender, lower prevalence of diabetes mellitus, higher lifetime alcohol and coffee consumption, and higher physical quality of life score. Silymarin was also modestly associated with a lower AST/ALT ratio and alkaline phosphatase levels and higher ALT, albumin levels, and prevalence of oesophageal varices ($P < 0.05$ for all). No association was observed for age, treatment group, patient cohort, body mass index, mental summary SF-36 score, serum AST, bilirubin, platelets, prothrombin time, HCV genotype or log RNA level, hepatic cirrhosis, collagen content, steatosis grade, or Ishak inflammation score.

At baseline, 621 patients had fibrosis and 428 patients had cirrhosis. During 4758 person-years of follow-up (median: 5.5 years per patient, interquartile range: 3.0–6.6 years), 384 patients had a two point increase in fibrosis score (TPI) from baseline or had a clinical outcome for liver disease. Combining these endpoints, we observed an inverse association between baseline silymarin use and liver disease progression (Table 2). In crude

models, the relative risk (RR) associated with former use of silymarin was 0.87 (95% CI: 0.66–1.15), whereas the RR for current use was 0.73 (95% CI: 0.54–0.98; P -trend across categories = 0.029). Upon stratification by outcome, current silymarin use was associated with less TPI (RR for current vs. never use of silymarin, 0.54, 95% CI: 0.32–0.93; P -trend = 0.015), but had no association with clinical outcomes (RR for current vs. never, 0.86, 0.61–1.20; P -trend = 0.42). Multivariate adjustment for age, education, race/ethnicity, gender, lifetime alcohol use, diabetes, coffee intake, ever use of other herbal products besides silymarin, mental and physical quality of life scores, baseline cirrhosis, AST/ALT ratio, albumin, platelets, bilirubin and oesophageal varices only modestly affected risk estimates. After multivariate adjustment, the RR for current vs. never use of silymarin was 0.57 (95% CI: 0.33–1.00; P -trend = 0.042) for TPI and 1.09 (95% CI: 0.77–1.56; P -trend = 0.89) for clinical outcomes.

Duration of silymarin use, prior to baseline, was also assessed. Compared to never users, patients who used silymarin for up to the median duration (16.6 months) had an RR for TPI of 0.93 (0.58–1.51), whereas patients who used silymarin for greater than the median duration had an RR of 0.51 (95% CI: 0.30–0.90; P -trend = 0.026). The RRs for clinical outcomes for the same two categories of silymarin use were 0.86 (95% CI: 0.61–1.21) and 0.94 (95% CI: 0.66–1.35; P -trend = 0.57) respectively (data not shown in table).

In addition to silymarin use at baseline, we examined silymarin use over the course of the study. At the time of the second biopsy, three and a half years after randomisation, 69% of baseline users continued to use silymarin (88/128), whereas only 3% of baseline non-users (15/477) had started use. The risk of TPI among patients with fibrosis who continued to use silymarin throughout the study was 0.55 (95% CI: 0.29–1.03; 68 patients, 11 events), whereas the risk of TPI among patients who stopped using silymarin during follow-up was 0.66 (95% CI: 0.23–1.87; 20 patients, 4 events) (data not shown in table).

Silymarin was the most commonly used herbal product in the HALT-C trial. Fourteen percent of patients used an herbal product other than silymarin ($n = 142$). Use of a nonsilymarin herbal product had no association with either TPI (0.92, 95% CI: 0.57–1.48) or clinical outcomes (0.87, 95% CI: 0.60–1.25) (data not shown in table).

Among those with fibrosis, comparing current users of silymarin with never users, the RR for TPI was 0.19 (95% CI: 0.02–2.05; 16 events) for patients with an Ishak

Table 1 | Baseline demographic, clinical and laboratory characteristics of 1049 participants of the HALT-C trial by category of silymarin use

Variables	Never	Former	Current	P for trend*
Number in cohort, <i>n</i> (%)	701 (66.8)	178 (17.0)	170 (16.2)	
Duration of silymarin use, months, median (IQR)	0	6 (3-12)	35 (20-53)	
Age, years, median (IQR)	50 (46-54)	48 (45-53)	50 (47-53)	0.52
Gender, female, <i>n</i> (%)	216 (70.8)	52 (17.1)	37 (12.1)	0.027
Race/ethnicity, Caucasian, <i>n</i> (%)	474 (63.1)	134 (17.8)	143 (19.0)	0.001
Treatment group, <i>n</i> (%)	337 (65.3)	93 (18.0)	86 (16.7)	0.41
Source of patient randomised				
Lead-in nonresponder	431 (65.1)	122 (18.4)	109 (16.5)	0.11
Lead-in breakthrough or relapse	97 (64.2)	27 (17.9)	27 (17.9)	
Express	173 (73.3)	29 (12.3)	34 (14.4)	
Education†, Completed college, <i>n</i> (%)	164 (60.1)	51 (18.7)	58 (21.3)	<.0001
Lifetime alcohol consumption, # of drinks, Median (IQR)	6758 (972-21,168)	7622 (1850-20,398)	9580 (1644-27,512)	0.035
Coffee intake,† drinks/day, Median (IQR)	1 (0.03-2)	1 (0.3-2)	1 (0.3-2)	0.023
Body Mass Index, Median (IQR)	29.3 (26.3-32.8)	29.4 (26.3-32.8)	28.5 (25.8-31.8)	0.23
Diabetes, Glucose ≥126 mg/dL, <i>n</i> (%)	186 (73.2)	36 (14.2)	32 (12.6)	0.016
Mental summary score from SF-36,† Median (IQR)	53 (46-57)	51 (45-56)	53 (47-57)	0.25
Physical summary score from SF-36,† Median (IQR)	47 (35-54)	47 (37-54)	50 (41-54)	0.038
AST, U/L, Median (IQR)	70 (50-102)	72 (54-124)	71 (49-113)	0.24
ALT, U/L, Median (IQR)	82 (57-123)	94 (67-140)	90 (60-139)	0.009
AST/ALT Ratio, Median (IQR)	0.82 (0.69-1.04)	0.79 (0.64-1.00)	0.80 (0.64-0.94)	0.004
Albumin, g/dL, Median (IQR)	3.9 (3.6-4.1)	3.9 (3.6-4.1)	4.0 (3.7-4.2)	0.018
Alk. Phos. U/L, Median (IQR)	90 (71-118)	89 (71-113)	84 (68-106)	0.012
Bilirubin, mg/dL, Median (IQR)	0.7 (0.5-0.9)	0.7 (0.5-0.9)	0.8 (0.5-1.0)	0.40
Platelets, 1000/mL, Median (IQR)	159 (116-208)	161 (114-205)	158 (119-197)	0.42
Prothrombin time, INR, Median (IQR)	1.0 (1.0-1.1)	1.0 (1.0-1.1)	1.0 (1.0-1.1)	0.49
Log ₁₀ HCV RNA level, Median (IQR)	6.5 (6.1-6.8)	6.5 (6.2-6.8)	6.6 (6.2-6.8)	0.58
HCV genotype 1, <i>n</i> (%)	649 (66.3)	167 (17.1)	163 (16.7)	0.12
Cirrhosis on biopsy, <i>n</i> (%)	280 (65.4)	74 (17.3)	74 (17.3)	0.38
Hepatic steatosis, ≥Grade 2	279 (64.0)	88 (20.2)	69 (15.8)	0.38
Ishak inflammation score, Median (IQR)	7 (6-9)	7 (6-9)	8 (6-9)	0.67
Oesophageal varices†, <i>n</i> (%)	158 (60.5)	52 (19.9)	51 (19.5)	0.021
Collagen content†, Median (IQR)	0.04 (0.02-0.08)	0.04 (0.02-0.09)	0.04 (0.02-0.07)	0.36

IQR: interquartile range; AST, aspartate aminotransferase; ALT, alanine aminotransferase; Alk. Phos., alkaline phosphatase; HCV, hepatitis C virus.

* Mantel-Haenszel test for trend for categorical variables. Jonckheere-Terpstra test for trend for continuous variables.

† Data not available for all participants: coffee was available for 791 participants; collagen content for 558 patients; education available for 1045 participants; oesophageal varices for 1016 participants; SF-36 scores for 1043 participants.

Table 2 | Association of silymarin use with liver disease progression in 1049 participants of the HALT-C trial

	Categories	Non-users	Former users	Current users	P-trend
TPI + clinical outcomes*	Cohort, <i>n</i> (%)	661 (66.6)	165 (16.6)	167 (16.8)	
	Case, <i>n</i> (%)	271 (70.6)	60 (15.6)	53 (13.8)	
	Crude, RR (95% CI)	1.00 (Ref)	0.87 (0.66–1.15)	0.73 (0.54–0.98)	0.029
	Multivariate #1,† RR (95% CI)	1.00 (Ref)	0.89 (0.67–1.18)	0.73 (0.54–0.99)	0.038
	Multivariate #2,‡ RR (95% CI)	1.00 (Ref)	0.74 (0.56–0.99)	0.84 (0.62–1.13)	0.089
TPI	Cohort, <i>n</i> (%)	368 (67.3)	91 (16.6)	88 (16.1)	
	Case, <i>n</i> (%)	116 (76.3)	21 (13.8)	15 (9.9)	
	Crude, RR (95% CI)	1.00 (Ref)	0.75 (0.47–1.19)	0.54 (0.32–0.93)	0.015
	Multivariate #1,† RR (95% CI)	1.00 (Ref)	0.75 (0.47–1.20)	0.56 (0.32–0.97)	0.025
	Multivariate #2,‡ RR (95% CI)	1.00 (Ref)	0.82 (0.51–1.32)	0.57 (0.33–1.00)	0.042
Clinical outcomes	Cohort, <i>n</i> (%)	701 (66.8)	178 (17.0)	170 (16.2)	
	Case, <i>n</i> (%)	187 (68.3)	46 (16.8)	41 (15.0)	
	Crude, RR (95% CI)	1.00 (Ref)	1.00 (0.73–1.38)	0.86 (0.61–1.20)	0.42
	Multivariate #1,† RR (95% CI)	1.00 (Ref)	1.07 (0.77–1.48)	0.89 (0.63–1.26)	0.65
	Multivariate #2,‡ RR (95% CI)	1.00 (Ref)	0.77 (0.54–1.08)	1.09 (0.77–1.56)	0.89

TPI, two point increase in Ishak fibrosis score; RR, relative risk; CI, confidence interval.

* Excluding 56 participants with fibrosis at baseline who did not receive follow-up biopsies or have a clinical outcome.

† Adjusted for continuous age, lifetime alcohol use and coffee intake along with categorical variables for education (high school or less, some post high school, completed college), race/ethnicity (Caucasian, African American, Hispanic and other), gender, diabetes and ever use of other herbal products besides silymarin, such as green tea, garlic, ginseng and Echinacea.

‡ Additionally adjusted for continuous mental and physical SF-36 quality of life scores, AST/ALT ratio, albumin, platelets, bilirubin, as well as categorical variables for cirrhosis status at baseline and presence or absence of oesophageal varices.

score of 2 at baseline, 0.48 (95% CI: 0.22–1.04; 87 events) for patients with an Ishak score of 3 at baseline, and 1.04 (95% CI: 0.37–2.90; 49 events) for patients with an Ishak score of 4 at baseline (data not shown in table). For those with fibrosis at baseline, we also examined the distribution of Ishak scores at year 1.5 and year 3.5 protocol biopsies. The distribution of Ishak scores was similar between former and never silymarin users for both biopsies ($P > 0.30$). For current silymarin users vs. never silymarin users, P -values for differences in the distribution of Ishak scores were 0.097 and 0.0059, for year 1.5 and year 3.5 biopsies respectively (Figure 1).

No association for clinical outcomes was found for those with either fibrosis or cirrhosis at baseline (RR for current use vs. never use, 1.36, 95% CI: 0.74–2.50, P -trend = 0.32, 98 events and 0.97, 95% CI: 0.61–1.53, P -trend = 0.41, 176 events, for fibrosis and cirrhosis respectively). The association between silymarin and clinical outcomes was also similar for outcomes occurring during years zero through four (RR for current vs. never use, 1.20, 95% CI: 0.78–1.86; P -trend = 0.85, 173 events) and five through eight of follow-up (0.88, 95% CI: 0.47–

1.63; P -trend = 0.88, 101 events) (data not shown in table). Kaplan–Meier curves for clinical outcomes among current, former and never users of silymarin were similar (Figure 2; $P = 0.657$). In a secondary analysis of 88 incident cases of HCC, compared to never use, the RR for former and current users was 1.15 (95% CI: 0.62–2.13) and 1.60 (95% CI: 0.93–2.76) respectively. This possible effect was restricted to events occurring during the first 4 years (HR: 1.96, 95% CI: 0.95–4.05; 47 events), but not years five-eight of follow-up (HR: 1.26, 95% CI: 0.54–2.94; 41 events).

Finally, we examined the association between silymarin use and biopsy collagen content as measured by morphometric image analysis (Table 3). The collagen content of each study biopsy appeared generally similar in former and never users of silymarin. But, the study biopsies of baseline silymarin users tended to have a lower collagen content than study biopsies of never users. For example, the mean collagen content on the year 3.5 biopsy was 0.071 (standard deviation = 0.069) among current silymarin users and 0.090 (standard deviation = 0.085) among never users, P -value = 0.061. The

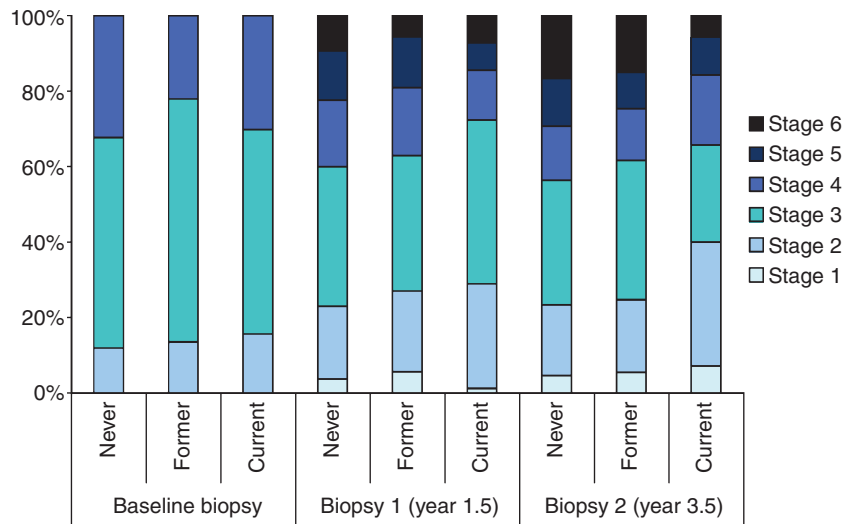


Figure 1 | Distribution at each biopsy for 621 patients without cirrhosis at baseline (Ishak score of 2, 3, or 4). Repeat protocol biopsies were performed 1.5 years (Biopsy 1, 524 patients) and 3.5 years after randomisation (Biopsy 2, 443 patients). The *P*-values for the distribution of Ishak scores of former vs. never silymarin users were 0.086, 0.30 and 0.47 for baseline, biopsy 1 and biopsy 2 respectively. For current silymarin users vs. never silymarin users, *P*-values for the distribution of Ishak scores at each biopsy were 0.42, 0.097 and 0.0059 respectively.

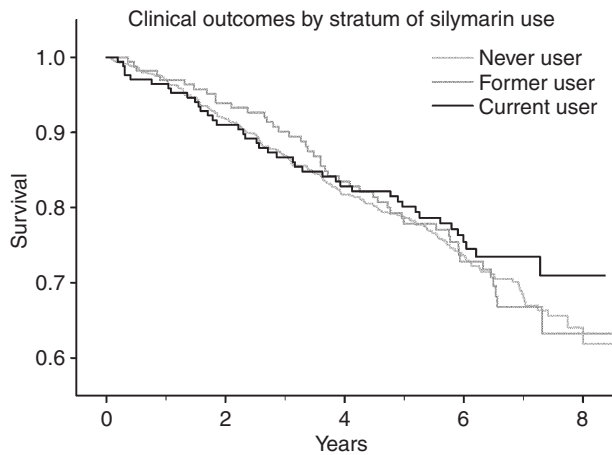


Figure 2 | Kaplan-Meier survival analysis of time to first clinical outcome by stratum of baseline silymarin use (never, former, and current; *P* = 0.657).

overall *P*-value comparing the change in collagen content across repeated biopsies in baseline silymarin users relative to never users was 0.037. After stratification by baseline cirrhosis status, the association between silymarin use with change in collagen content across repeated study biopsies persisted in both patients with fibrosis (overall *P*-value = 0.034) and those with cirrhosis (overall *P*-value = 0.011) at baseline.

DISCUSSION

In a large prospective cohort of individuals with advanced hepatitis C-related chronic liver disease, no clinical benefit was found for current silymarin use at baseline. In addition, we observed a nonsignificant increase in HCC risk among current silymarin users, which was present only in the first 4 years of follow-up. Baseline silymarin use was associated with less liver disease progression as measured by a two-point increase in Ishak fibrosis score as well as in the distribution of fibrosis scores in follow-up biopsies. A dose-response with duration of use was observed. Current use of silymarin at baseline, but not former use prior to baseline, was associated inversely with biopsy collagen content, regardless of whether patients had fibrosis or cirrhosis.

Silymarin has been used to treat liver disease for thousands of years.^{6, 27} Furthermore, results from animal, *in vitro* and clinical studies suggest that silymarin has possible anti-inflammatory,⁹⁻¹¹ anti-viral,¹¹⁻¹⁴ antioxidant^{10, 15} and antifibrotic effects.^{10, 16, 17} Yet, few clinical and observational studies have evaluated the effect of silymarin on liver disease progression and clinical outcomes in humans. Previous studies had small size, limited power to detect associations, and yielded mixed results.^{6, 27} For example, one trial of 170 patients with alcohol-related liver disease showed an effect of silymarin

Table 3 | Morphometric collagen content at each biopsy by stratum of silymarin use

Silymarin use	Baseline biopsy			Biopsy 1 (year 1.5)			Biopsy 2 (year 3.5)			Overall P-value across repeated biopsies†
	Mean (s.d.)	n	P-value relative to never users*	Mean (s.d.)	n	P-value relative to never users*	Mean (s.d.)	n	P-value relative to never users*	
All patients										
Never (n = 701)	0.061 (0.061)	361	Referent group	0.088 (0.079)	358	Referent group	0.090 (0.085)	271	Referent group	Referent group
Former (n = 178)	0.062 (0.058)	113	0.82	0.089 (0.092)	100	0.81	0.077 (0.087)	69	0.15	0.93
Current (n = 170)	0.054 (0.056)	84	0.17	0.068 (0.053)	92	0.10	0.071 (0.069)	69	0.061	0.037
Fibrosis at baseline										
Never (n = 421)	0.039 (0.036)	231	Referent group	0.070 (0.064)	239	Referent group	0.069 (0.065)	182	Referent group	Referent group
Former (n = 104)	0.037 (0.028)	71	0.95	0.070 (0.088)	64	0.38	0.064 (0.072)	48	0.41	0.53
Current (n = 96)	0.031 (0.027)	46	0.15	0.063 (0.057)	58	0.25	0.043 (0.036)	42	0.018	0.034
Cirrhosis at baseline										
Never (n = 280)	0.101 (0.075)	130	Referent group	0.124 (0.092)	119	Referent group	0.134 (0.103)	89	Referent group	Referent group
Former (n = 74)	0.105 (0.070)	42	0.60	0.122 (0.089)	36	0.97	0.107 (0.110)	21	0.14	0.98
Current (n = 74)	0.081 (0.069)	38	0.052	0.077 (0.045)	34	0.023	0.115 (0.086)	27	0.48	0.011

s.d., standard deviation.

Assessment of morphometric collagen content was limited to biopsies with at least 10 mm² of tissue, including 558 biopsies at baseline, 550 biopsies at year 1.5, and 409 biopsies at year 3.5.

* Mann-Whitney test for the difference between mean collagen content in former or current silymarin users relative to never users.

† P-value for the overall effect of silymarin use on morphometric collagen content across repeated biopsies was assessed by repeated-measures analysis of variance assuming an autoregressive covariance structure. Time (baseline, biopsy 1, and biopsy 2) and silymarin use were included in the models as fixed effects.

on survival,²⁸ whereas a second trial of 200 patients showed similar survival rates in the randomised and control arms.²⁹ Even fewer data are available for hepatitis C-related liver disease. Data from an Egyptian randomised trial of 141 patients showed no effect for silymarin on outcomes.^{30, 31}

It is not clear why silymarin was associated with a reduction in rate of fibrosis progression, but not with clinical outcomes in our study. One possibility is that in order to exert an effect, silymarin must be used early in the disease progression process. In support of this hypothesis, silymarin seemed to have an effect on histological progression if patients had an Ishak score of 2 or 3 at baseline, but no effect on individuals with a score of 4 at baseline. On the other hand, silymarin had no effect on clinical outcomes for individuals with either cirrhosis or fibrosis at baseline, or for outcomes occurring during the first 4 years, or years five-eight of follow-up. It remains possible, however, that follow-up was too short to see an effect on clinical outcomes.

Fibrosis progression is not the sole determinant of subsequent decompensation or complications of portal hypertension. As such, it is also possible that silymarin does not have a beneficial effect on other determinants of clinical outcomes.³² Alternatively, differences between histological progression and clinical outcomes could simply reflect chance.

Strengths of our study include assessment of silymarin use before disease progression, the large number of patients with advanced hepatitis C-related liver disease, comprehensive assessment of clinical, demographic and lifestyle information, and careful assessment of clinical and histological outcomes. The major limitation was a complete lack of information on the amount of silymarin patients used per day. We also lacked information on how silymarin was prepared. Patients in the HALT-C trial probably used many different dosages and formulations of silymarin and even for individual patients, preparations probably varied day by day and week by week. Furthermore, it is unlikely that patients would have ingested pharmacological doses of silymarin as have shown effect *in vitro*, clinical, and animal studies. For example, a recent study of 36 patients observed an effect of intravenously (i.v.) administered silymarin (as silibinin) on hepatitis C viral level,¹⁴ although a study with similar dosing of orally administered silymarin showed no effect.³³ Most likely, patients in HALT-C used less silymarin than those in the i.v. study. Further complicating interpretation is that the pharmacokinetics of silymarin may be altered by fibrosis. A recent study

administered a standard silymarin dose to cirrhotics and healthy volunteers. In response to silymarin treatment, serum flavonolignans were higher in the cirrhotic volunteers.³⁴ Finally, not all study biopsies were large enough to have morphometric analysis performed, a potential source of selection bias. Indeed, patients with cirrhosis were less likely to receive all biopsies.²¹ Yet, as we observed an apparent inverse association between silymarin use and collagen content, such a bias, if anything, would probably attenuate the observed association between silymarin use and collagen content.

In the HALT-C trial, use of silymarin was associated with Caucasian race, completing college and a higher SF-36 physical quality of life score, suggesting that silymarin use might be a marker for a large number of other lifestyle factors. We adjusted our risk estimates for these and other possible confounders. After adjustment, risk estimates were only modestly altered. In addition, the observed effect of silymarin does not simply reflect a propensity to use herbal products. Using herbal products, other than silymarin, had no association with either histological progression or clinical outcomes in our study. Nevertheless, as an observational study, the inverse association observed between silymarin use and histological progression could reflect another exposure or chance. We did not have any information on brand or dosage of silymarin. However, this limitation is reflective of the difficulty in detailing patient behaviour outside controlled studies. Many, if not most, patients with currently incurable liver disease seek alternative, unapproved therapies that cannot be easily quantified, yet deserve evaluation.

In summary, among individuals with advanced hepatitis-C-associated liver disease, we observed an inverse association between silymarin use and the progression of liver disease from fibrosis to cirrhosis, but no evidence for an effect on clinical outcomes. As our results are from an observational study, it is possible that the observed beneficial effect on liver disease progression is due to chance. Future studies with a comprehensive assessment of silymarin dose are needed to replicate these findings. Nevertheless, our results provide support for conducting additional studies of silymarin, including intervention trials with defined dosage regimens and standard silymarin product. Such studies would be most appropriate for patients who have not responded to or are not candidates for anti-viral therapy and have limited other treatment options. Importantly, our results do not support the use of *ad hoc* dosing of silymarin by patients with chronic liver disease.

ACKNOWLEDGEMENTS

Declaration of personal interests: None. In addition to the authors of this manuscript, the following individuals were instrumental in the planning, conduct and/or care of patients enrolled in this study at each of the participating institutions as follows:

University of Massachusetts Medical Center, Worcester, MA: (Contract N01-DK-9-2326) Gyongyi Szabo, MD, Barbara F. Banner, MD, Maureen Cormier, RN, Donna Giansiracusa, RN.

University of Connecticut Health Center, Farmington, CT: (Grant M01RR-06192) Herbert L. Bonkovsky, MD, Gloria Borders, RN, Michelle Kelley, RN, ANP.

Saint Louis University School of Medicine, St Louis, MO: (Contract N01-DK-9-2324) Adrian M. Di Bisceglie, MD, Bruce Bacon, MD, Brent Neuschwander-Tetri, MD, Elizabeth M. Brunt, MD, Debra King, RN.

Massachusetts General Hospital, Boston, MA: (Contract N01-DK-9-2319, Grant M01RR-01066; Grant 1 UL1 RR025758-01, Harvard Clinical and Translational Science Center) Jules L. Dienstag, MD, Raymond T. Chung, MD, Andrea E. Reid, MD, Atul K. Bhan, MD, Wallis A. Molchen, David P. Lundmark.

University of Colorado Denver, School of Medicine, Aurora, CO: (Contract N01-DK-9-2327, Grant M01RR-00051, Grant 1 UL1 RR 025780-01), Gregory T. Everson, MD, Thomas Trouillot, MD, Marcelo Kugelmas, MD, S. Russell Nash, MD, Jennifer DeSanto, RN, Carol McKinley, RN.

University of California - Irvine, Irvine, CA: (Contract N01-DK-9-2320, Grant M01RR-00827) Timothy R. Morgan, MD, John C. Hoefs, MD, John R. Craig, MD, M. Mazen Jamal, MD, MPH, Muhammad Sheikh, MD, Choon Park, RN.

University of Texas Southwestern Medical Center, Dallas, TX: (Contract N01-DK-9-2321, Grant M01RR-00633, Grant 1 UL1 RR024982-01, North and Central Texas Clinical and Translational Science Initiative) William M. Lee, MD, Thomas E. Rogers, MD, Peter F. Malet, MD, Janel Shelton, Nicole Crowder, LVN, Rivka Elbein, RN, BSN, Nancy Liston, MPH.

University of Southern California, Los Angeles, CA: (Contract N01-DK-9-2325, Grant M01RR-00043) Karen L. Lindsay, MD, MMM, Sugantha Govindarajan, MD, Carol B. Jones, RN, Susan L. Milstein, RN.

University of Michigan Medical Center, Ann Arbor, MI: (Contract N01-DK-9-2323, Grant M01RR-00042, Grant 1 UL1 RR024986, Michigan Center for Clinical and Health Research) Anna S. Lok, MD, Robert J.

Fontana, MD, Joel K. Greenson, MD, Pamela A. Richtmyer, LPN, CCRC, R. Tess Bonham, BS.

Virginia Commonwealth University Health System, Richmond, VA: (Contract N01-DK-9-2322, Grant M01RR-00065) Mitchell L. Shiffman, MD, Richard K. Sterling, MD, MSc, Melissa J. Contos, MD, A. Scott Mills, MD, Charlotte Hofmann, RN, Paula Smith, RN.

Liver Diseases Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD: Marc G. Ghany, MD, T. Jake Liang, MD, David Kleiner, MD, PhD, Yoon Park, RN, Elenita Rivera, RN, Vanessa Haynes-Williams, RN.

National Institute of Diabetes and Digestive and Kidney Diseases, Division of Digestive Diseases and Nutrition, Bethesda, MD: Patricia R. Robuck, PhD, Jay H. Hoofnagle, MD.

University of Washington, Seattle, WA: (Contract N01-DK-9-2318) David R. Gretch, MD, PhD, Minjun Chung Apodaca, BS, ASCP, Rohit Shankar, BC, ASCP, Natalia Antonov, M. Ed.

New England Research Institutes, Watertown, MA: (Contract N01-DK-9-2328) Kristin K. Snow, MSc, ScD, Anne M. Stoddard, ScD, Margaret C. Bell, MS, MPH.

Armed Forces Institute of Pathology, Washington, DC: Fanny Monge, Michelle Parks.

Data and Safety Monitoring Board Members: (Chair) Gary L. Davis, MD, Guadalupe Garcia-Tsao, MD, Michael Kutner, PhD, Stanley M. Lemon, MD, Robert P. Perrillo, MD.

Declaration of funding interests: This study was funded in part by the National Institute of Diabetes & Digestive & Kidney Diseases (contract numbers are listed below). Additional support was provided by the National Institute of Allergy and Infectious Diseases (NIAID), the National Cancer Institute, the National Center for Minority Health and Health Disparities and by General Clinical Research Center and Clinical and Translational Science Center grants from the National Center for Research Resources, National Institutes of Health (grant numbers are listed in the Acknowledgement). This research was also supported in part by the Intramural Research Program of the National Cancer Institute. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Center for Research Resources or the National Institutes of Health. Additional funding to conduct this study was supplied by Hoffmann-La Roche, Inc., through a Cooperative Research and Development Agreement (CRADA) with the National Institutes of Health.

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