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Statin Use is Associated with a Reduced Risk of Fibrosis Progression in Chronic Hepatitis C

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Abstract

Background & Aims—Therapies that slow fibrosis progression in chronic liver disease are needed. Animal models demonstrate that statins prevent progression of hepatic fibrosis, but human data is lacking. We evaluated the association between statins and fibrosis progression in the HALT-C Trial cohort.

Methods—Subjects with chronic hepatitis C (CHC) and advanced hepatic fibrosis underwent serial liver biopsies over 3.5 years. The primary outcome was a ≥ 2-point increase in Ishak fibrosis score on at least one of two serial biopsies. We used complementary log-log regression analysis to assess the association between statins and fibrosis progression among subjects without baseline cirrhosis.

Results—Fibrosis progression occurred in 3/29 (10%) statin users and 145/514 (29%) non-users. The unadjusted hazard ratio (HR) for fibrosis progression among statin users compared to non-users was 0.32 (95% CI 0.10-0.99). This association remained significant after adjusting for established predictors of histological outcome, including body mass index, platelets and hepatic steatosis (adjusted HR 0.31; 95% CI 0.10-0.97). The mean change in Ishak fibrosis score over the

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3.5 year study period was -0.34 (SE 0.18) for statin users compared to +0.42 (SE 0.07) for non-users (p= 0.006 after adjustment for baseline fibrosis score).

Conclusions—Statin use is associated with a reduced risk of fibrosis progression in advanced CHC. Our findings suggest a potential role for statins in preventing liver disease progression.

Keywords

cirrhosis; hepatitis C virus; lipid lowering agent

Introduction

Hepatitis C virus (HCV) is one of the most common causes of chronic liver disease and the leading indication for liver transplantation worldwide [1, 2]. Chronic hepatitis C (CHC) leads to cirrhosis and hepatocellular carcinoma (HCC). Estimates suggest that over a period of twenty to thirty years, cirrhosis will develop in 10% to 25% of patients with CHC, and HCC in 1% to 5% [2]. Although the success of HCV therapy now stands to improve with the introduction of direct-acting antiviral medications, there are still many patients with advanced fibrosis in whom disease progression may nevertheless occur, despite successful viral clearance.

Statins, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, have garnered attention for their pleiotropic effects. There is mounting evidence that statins offer chemoprevention against many malignancies, including HCC [3-8]. Via both HMG-CoA dependent and independent pathways, statins inhibit cell growth, decrease proteolysis and block tumor cell spread [8-13]. They also exert powerful antiproliferative, antiangiogenic, proapoptotic and immunomodulatory effects [14-16].

In contrast, data regarding the antifibrogenic actions of statins is more limited. In animal models, statins block activation of hepatic myofibroblasts, inducing apoptosis and preventing both proliferation of hepatic stellate cells (HSCs) and their production of collagens [13, 15-19]. Reports in humans have been based largely upon retrospective studies of laboratory markers of hepatotoxicity, and are limited by small sample sizes, lack of appropriate controls or histological data from liver biopsy, which remains the gold standard for the assessment of fibrosis [20-22].

We sought to evaluate the association between statin use and histologic fibrosis progression using the Hepatitis C Antiviral Long-term Treatment against Cirrhosis (HALT-C) Trial cohort. The HALT-C Trial was a multicenter, randomized controlled trial, designed to evaluate the benefit of long-term peginterferon therapy in patients with CHC and advanced fibrosis who had previously failed to respond to antiviral therapy [23]. This cohort is ideal for assessing the antifibrotic and chemopreventive properties of statins due to its large sample size, prospective and randomized study design, long length of follow-up and the availability of serial liver biopsy specimens.

Patients and Methods

The HALT-C Cohort

The HALT-C Trial included 1,050 subjects with CHC who had previous non-response to standard interferon therapies and advanced hepatic fibrosis on liver biopsy (Ishak fibrosis score ≥3). Subjects with evidence of hepatic decompensation, other concurrent cause of liver disease, or HCC, were excluded. Although entry into the trial was limited to subjects with Ishak fibrosis stage ≥3, staging of biopsies by local pathologists at individual sites was reclassified if a central reassessment by all study pathologists resulted in a different consensus. In addition, subjects with a baseline Ishak fibrosis score of 2 were eligible for enrollment if a prior liver biopsy had shown fibrosis score ≥3. Thus, among the 1050 randomized subjects, 72 had baseline Ishak fibrosis scores of 2 [23-25].

During the lead-in phase of the trial, all patients were treated with pegylated interferon alfa-2a and ribavirin for at least 24 weeks before undergoing randomization. Patients with detectable serum HCV RNA levels at treatment week 20 were assigned for the next 3.5 years to either the maintenance-therapy group (90 μg of peginterferon alfa-2a weekly, without ribavirin) or the untreated control group. Patients with undetectable serum HCV RNA at week 20 continued therapy for up to an additional 28 weeks. If HCV RNA was detected again after week 20, the patient was offered the opportunity to undergo randomization in the controlled phase of the trial. “Express” patients who were non-responders to peginterferon/ribavirin outside the HALT-C trial were also directly enrolled into the randomized phase of the trial.

Because clinical outcomes, including fibrosis progression, occurred in an indistinguishable proportion of treatment and control subjects [23, 25], we included in the analysis the entire randomized population with baseline non-cirrhotic fibrosis who had at least one of two serial biopsies (n=547).

Ascertainment of outcomes

Patients were evaluated every 3 months during the 3.5 years of the randomized trial and every 6 months thereafter unless consent was withdrawn or a study-ending clinical outcome including liver transplantation occurred. For lead-in patients, the baseline liver biopsy was performed at least 2 months following the last course of interferon therapy and within 12 months of enrollment. For express patients, a liver biopsy must have been performed within 18 months before randomization and at least 8 weeks after the end of the previous course of peginterferon and ribavirin. Liver biopsies were repeated at 1.5 and 3.5 years after randomization. All biopsies were reviewed by an expert panel of hepatopathologists; inflammation was graded according to an 18-point histology activity index (HAI), and fibrosis was staged according to the Ishak scale [24]. Hepatic steatosis was graded on the percentage of hepatocytes containing fat as 0 (<1%), 1 (1%-5%), 2 (5%-33%), 3 (33%-67%), and 4 (>67%). A histological outcome was defined as an increase in Ishak Fibrosis score by ≥ 2 points on at least one of the follow-up liver biopsies, 1.5 or 3.5 years after randomization (from Ishak Fibrosis score 2 to 4-6, score 3 to 5-6, or score 4 to 6).

Statin use

Use of statin medications was elicited as part of a comprehensive medication history obtained prior to enrollment and at each subsequent follow-up visit. Continuous statin use was defined as use of a statin medication from baseline throughout the study observation period. Four patients, who initiated statins during the study observation period, one at day 261, one at day 564, one at day 639, and one at day 1166, were excluded from the analysis. Statin dose information was not available.

Statistical Analysis

Baseline demographics and clinical characteristics of all eligible HALT-C patients were assessed. Univariate comparisons of the distribution of baseline variables between continuous statin users and non-users were performed using the Student's t-test and the Wilcoxon rank sum test for continuous data, and the Chi-square and Fischer's exact test for categorical data.

For time to histological progression, complementary log-log regression analysis was used, given that the time-to-event analysis was grouped at 1.5 and 3.5 years post-randomization. For the multivariable model, we selected previously established independent predictors of histological outcome: baseline body mass index (BMI), platelet count and hepatic steatosis [25]. Given the differential distribution of age, diabetes, race, metformin use, and alanine aminotransferase (ALT) among statin users and non-users, we included these variables in multivariable model in an additional sensitivity analysis. We also performed a univariate analysis of the association between non-statin lipid lowering agents and fibrosis progression to ensure that the association between statin use and fibrosis progression was independent of the lipid lowering properties of statins.

Change in Ishak fibrosis score, HAI, and ALT levels over the 3.5-year follow up period were analyzed using analysis of covariance, adjusting for baseline levels. Four hundred fifty-six patients had both baseline and 3.5-year biopsies, and were included in the change in fibrosis score and HAI analysis. Four hundred ninety patients had ALT levels measured at baseline and 3.5 years and were included in the change in ALT analysis. Linear regression was used to compare the change in log 10 HCV RNA over the study period, adjusting for randomization status, among statin users and non-users.

A 2-tailed P-value < 0.05 was considered statistically significant. SAS (Cary, NC) version 9.3 was used for statistical analyses.

Results

Table 1 shows the demographic, clinical and histological data of the patients with baseline non-cirrhotic fibrosis, according to continuous statin medication use. Twenty-nine patients used statins continuously during the 3.5 year study period while 514 patients did not take statins during the study observation period. Compared with non-users, continuous statin users were more likely to be older and of African American race, have diabetes, and use both metformin and other lipid lowering drugs. The baseline Ishak fibrosis scores and HAI scores were similar between the two groups. Continuous statin users did have lower baseline

ALT and aspartate aminotransferase (AST) levels than non-users although there was no significant difference in baseline AST:ALT ratio between the two groups (Table 1).

Fibrosis progression occurred in only 3/29 (10%) continuous statin users and 145/514 (29%) non-users. The unadjusted HR for fibrosis progression among continuous statin users compared to non-users was 0.32 (95% CI 0.10-0.99, p=0.048) (Table 2). After adjusting for established independent predictors of fibrosis progression, including platelet count, BMI, and hepatic steatosis [25], the association between statin use and fibrosis progression remained significant (HR 0.31; 95%CI 0.10-0.97; p=0.044).

When diabetes, race, age, ALT, and metformin use were added to the multivariable model, the HR for fibrosis progression among continuous statin users compared to non-users was 0.33 (95%CI 0.08-1.08; p=0.068). The use of other lipid lowering drugs was not associated with fibrosis progression (unadjusted HR=0.59; 95%CI 0.26-1.33; p=0.21). The use of metformin was also not associated with fibrosis progression (unadjusted HR=1.01; 95%CI 0.61-1.66; p=0.96).

The mean change in Ishak fibrosis score over the 3.5 year study period was -0.34 among continuous statin users and +0.42 among non-users (p=0.006 after adjusting for baseline Ishak fibrosis score) (Table 3). Because the change in Ishak fibrosis score is not truly a continuous variable, we also used ordinal logistic regression to analyze the change in Ishak fibrosis score. The odds ratio for a 1 point increase in fibrosis score over the 3.5 year study period was 0.38 (95%CI 0.19-0.78; p=0.009) for statin users compared to non-users. The mean change in HAI score was not significantly different between continuous statin users and non-users (mean change -1.27 and -0.52, respectively, p=0.15). The mean change in ALT over the study period was also not significantly different between the two groups (mean change -15.8 and -22.0 U/L, respectively, p=0.06). Additionally, the median log 10 decline in HCV RNA among statin users was -0.10 (IQR -0.38 to +0.16) and the median log 10 decline in HCV RNA among non-users was -0.14 (IQR -0.47 to +0.17), p=0.85. Adjusting for randomization to the treatment or placebo group did not alter these results (adjusted p value=0.99).

Discussion

Statins are among the most commonly prescribed medications worldwide. They have been shown to be safe in chronic liver disease [11, 22]. Mounting evidence has demonstrated their antiproliferative, antiangiogenic, anti-inflammatory and anti-neoplastic effects [6-8]. However, little is known about the role of statins in the natural history of fibrosis progression in humans [16-18, 26]. Using the HALT-C Trial cohort, we found an association between statin medication use and decreased risk of fibrosis progression. This association persisted even after adjusting for established predictors of fibrosis progression.

The HALT-C trial was designed to only include patients with advanced hepatic fibrosis (Ishak fibrosis score ≥3), therefore it is not surprising that baseline fibrosis scores were comparable between statin users and non-users. However, it is notable that despite similar

baseline values, the mean fibrosis score of continuous statin users decreased over the 3.5-year study period, whereas the mean fibrosis score of non-users increased.

Although statin users had lower mean baseline ALT levels than non-users, other markers of necroinflammation, such as HAI score and AST:ALT ratio, were similar among the two groups. Additionally, there was no statistically significant difference in the 3.5-year change in mean ALT level or HAI score when statin users and non-users were compared. This finding raises the possibility that statins may modulate fibrosis progression through pathways other than inflammation.

Given the frequent co-occurrence of diabetes and hyperlipidemia, it stands to reason that a high percentage of statin-users were also prescribed the anti-diabetic agent metformin. Like statins, metformin has been shown to possess anti-inflammatory and chemopreventive properties; it has been shown to inhibit hepatoma cell proliferation and induce cell cycle arrest at G0/G1 phase, via AMP-activated protein kinase and its upstream kinase, LKB1 [3, 15, 16]. Among the HALT-C cohort, however, we did not detect an association between metformin use and fibrosis progression. However, when age, race, diabetes, metformin, and ALT were added to the multivariable model with steatosis, BMI, and platelets, the significance of the association between statin use and decreased risk of fibrosis progression was borderline. We suspect that this borderline significance is related to the low number of statin users and limited power to detect the association within the larger multivariable model. Larger studies with a greater proportion of statin users will be required to confirm this relationship and validate our findings.

Liver fibrosis involves activation of HSCs, which undergo a phenotypic change from their quiescent state into proliferative and chemotactic myofibroblasts. HSC activation involves expression of α-smooth muscle actin (αSMA) and cytoskeletal reorganization with loss of lipid droplets. Myofibroblasts have increased gene expression of extracellular matrix proteins such as collagen, along with the profibrotic cytokines transforming growth factor-β (TGFβ), platelet-derived growth factor (PDGF) and connective tissue growth factor (CTGF). These cytokines stimulate the activation of additional HSCs, resulting in further activation and fibrogenesis [27]. An early *in vitro* study showed that the addition of simvastatin to human HSCs led to decreased cell growth, with a dose-dependent reduction in cellular thymidine uptake and inhibition of DNA synthesis. Addition of mevalonic acid overcame this effect, suggesting that the growth inhibition was a direct result of HMG-CoA reductase blockade [28].

In vivo, investigators have shown an antifibrotic effect of atorvastatin in a bile duct ligation (BDL) rat model characterized by rapidly progressing biliary fibrosis. Prophylactic and early therapy with atorvastatin significantly attenuated fibrosis, as assessed by hepatic hydroxyproline content, and myofibroblast accumulation, as assessed by hepatic αSMA expression. While later therapy did not result in significant fibrosis reduction, it was associated with decreased expression of profibrotic cytokines including TGFβ1, CTGF and PDGFβ-receptor mRNA levels. In addition, the HSCs were more quiescent, with decreased proliferation and apoptosis, suggesting that even in advanced disease, statins may induce senescence and attenuate hepatic fibrosis. Interestingly, aminotransferase levels, a surrogate

marker for hepatic inflammation, did not change with atorvastatin administration [17]. These findings in the BDL rat model concur with our findings in the HALT-C trial, particularly given that we found a significant decrease in mean Ishak fibrosis score but not ALT level over the HALT-C study period.

Statin-mediated modulation of fibrosis may also result from the beneficial effects of statins upon liver microcirculation. Activation of Kruppel-like factor 2 (KLF2), a vasoprotective transcriptional factor expressed in endothelium, lung, and lymphocytes, results in upregulation of vasodilator, antithrombotic and anti-inflammatory genes, including endothelial nitric oxide synthase and thrombomodulin. It also reduces expression of adhesion molecules, and inhibits oxidative stress-mediated cell injury and apoptosis [29-31]. Investigators recently studied the effect of statin administration on the expression of KLF2 and its vasoprotective target genes in carbon tetrachloride treated cirrhotic rats. Statin administration to liver sinusoidal endothelial cells (SEC) led to significant upregulation of KLF2 expression. In turn, statin-mediated KLF2 overexpression resulted in HSC quiescence, through a KLF2-nitric oxide-guanylate cyclasemediated paracrine mechanism [31]. Activated HSCs co-cultured with SEC previously treated with simvastatin exhibited a significant improvement in phenotype with decreased pro collagen I and αSMA [32]. Together these studies suggest clear potential mechanisms though which statins could abrogate liver fibrosis.

The antifibrotic benefits of statins may be especially pronounced in CHC. Statins inhibit the formation of lipid rafts and block the formation of geranylgeranylated F-box/leucine-rich repeat protein 2, both of which are necessary for HCV replication [33-35]. Statins may also synergistically improve viral response to hepatitis C therapy [36]. In vitro, statins have been shown to inhibit host factors that participate in HCV replication, resulting in significant anti-HCV activity [37-40] While statins may have an effect on HCV replication *in vitro*, the findings that baseline HCV RNA levels and the log 10 decline in HCV RNA levels over the study period were not significantly different between statin users and non-users argues against a meaningful *in vivo* antiviral effect of statins and, by extension, for an antiviral effect to have explained the observed difference in fibrosis progression. An antiviral effect for statins in some clinical trials has only been observed in conjunction with full dose peginterferon and ribavirin [41-43]. The use of statins alone or with low dose interferon in HALT-C, however, did not suffice to produce a meaningful antiviral effect.

Finally, statins have also been shown to inhibit the epidermal growth factor (EGF) signaling pathway [44, 45]. Ligands which activate the EGF receptor (EGFR) have been shown to increase HCV cellular entry [46]. Although the effect of statins on EGF signaling in hepatocytes has not yet been studied, experimental animal models have demonstrated that treatment with erlotinib, an EGFR tyrosine kinase inhibitor, reduces EGFR phosphorylation in HSC and also reduces the total number of activated HSC. Erlotinib use prevented the progression of cirrhosis and in some animals resulted in fibrosis regression [47].

Other human studies have suggested a beneficial role for statin administration in decreasing portal hypertension. In one analysis, administration of simvastatin led to an increase in the hepatosplanchnic output of nitric oxide products and a decrease in intrahepatic resistance

[48]. Additionally, in a randomized control trial, simvastatin decreased hepatic venous pressure gradient and improved liver perfusion in patients with cirrhosis. There was no significant increase in adverse events in patients receiving statins, supporting the safety of statin administration in chronic liver disease [49]. These findings raise the possibility that improvement in portal pressures could be linked to the antifibrotic effects of statins.

In addition to being the first to prospectively evaluate the impact of statin medications on hepatic fibrosis in humans, this study has several unique strengths including well-documented statin use recorded at serial study visits, a relatively long length of follow-up, and histological data obtained from serial liver biopsies.

Limitations of this study include the absence of precise information on statin dose and duration prior to study enrollment, as well as relatively high baseline Ishak fibrosis scores (≥3 in the majority). Conceivably, if statins exert their maximal antifibrotic effects early in the fibrosis process, this relationship could have been missed. Secondly, the HALT-C Trial was powered to assess the impact of long-term peginterferon alfa-2a use on a composite of fibrosis progression and clinical outcomes in 1,050 patients. Among those with baseline non-cirrhotic fibrosis there were only 29 statin users, with a relatively limited number of outcomes, which in turn limited our ability to detect and association between statin use and fibrosis in the larger multivariable model incorporating age, race, ALT, diabetes and metformin use, along with known predictors of fibrosis progression. These limitations, however, are outweighed by the strength of the study design and the quality of the data regarding statin exposure and subsequent histologic outcomes.

In conclusion, our findings demonstrate a significant reduction in the risk of fibrosis progression among statin users with advanced CHC. These results support a possible role for statins in the prevention of liver disease progression. Further studies with a larger proportion of statin users and pathologic endpoints are warranted. Such analyses will help to define the optimal timing of statin initiation, ideal duration of therapy, and the impact of statins on those with less severe fibrosis or other etiologies of liver disease.

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References

1. Armstrong GL, Wasley A, Simard EP, McQuillan GM, Kuhnert WL, Alter MJ. The prevalence of hepatitis C virus infection in the United States, 1999 through 2002. Annals of internal medicine. 2006; 144:705–714. [PubMed: 16702586]
2. Thomas DL, Seeff LB. Natural history of hepatitis C. Clinics in liver disease. 2005; 9:383–398. vi. [PubMed: 16023972]
3. Kisseleva T, Brenner DA. Anti-fibrogenic strategies and the regression of fibrosis. Best practice & research Clinical gastroenterology. 2011; 25:305–317. [PubMed: 21497747]

4. Demierre MF, Higgins PD, Gruber SB, Hawk E, Lippman SM. Statins and cancer prevention. Nature reviews Cancer. 2005; 5:930–942.
5. Welzel TM, Graubard BI, Zeuzem S, El-Serag HB, Davila JA, McGlynn KA. Metabolic syndrome increases the risk of primary liver cancer in the United States: a study in the SEER-Medicare database. Hepatology. 2011; 54:463–471. [PubMed: 21538440]
6. Bonovas S, Filioussi K, Tsavaris N, Sitaras NM. Statins and cancer risk: a literature-based meta-analysis and meta-regression analysis of 35 randomized controlled trials. Journal of clinical oncology: official journal of the American Society of Clinical Oncology. 2006; 24:4808–4817. [PubMed: 17001070]
7. Singh S, Singh PP, Singh AG, Murad MH, Sanchez W. Statins are associated with a reduced risk of hepatocellular cancer: a systematic review and meta-analysis. Gastroenterology. 2013; 144:323–332. [PubMed: 23063971]
8. Kuoppala J, Lamminpaa A, Pukkala E. Statins and cancer: A systematic review and meta-analysis. European journal of cancer. 2008; 44:2122–2132. [PubMed: 18707867]
9. Kalluri R, Neilson EG. Epithelial-mesenchymal transition and its implications for fibrosis. The Journal of clinical investigation. 2003; 112:1776–1784. [PubMed: 14679171]
10. Kisseleva T, Brenner DA. Mechanisms of fibrogenesis. Experimental biology and medicine. 2008; 233:109–122. [PubMed: 18222966]
11. Schuppan D, Afdhal NH. Liver cirrhosis. Lancet. 2008; 371:838–851. [PubMed: 18328931]
12. Iwaisako K, Brenner DA, Kisseleva T. What's new in liver fibrosis?. The origin of myofibroblasts in liver fibrosis. Journal of gastroenterology and hepatology. 2012; 27(Suppl 2):65–68. [PubMed: 22320919]
13. Shirin H, Sharvit E, Aeed H, Gavish D, Bruck R. Atorvastatin and rosuvastatin do not prevent thioacetamide induced liver cirrhosis in rats. World journal of gastroenterology: WJG. 2013; 19:241–248. [PubMed: 23345947]
14. Sun HY, Singh N. Antimicrobial and immunomodulatory attributes of statins: relevance in solid-organ transplant recipients. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2009; 48:745–755. [PubMed: 19193110]
15. Wu J, Wong WW, Khosravi F, Minden MD, Penn LZ. Blocking the Raf/MEK/ERK pathway sensitizes acute myelogenous leukemia cells to lovastatin-induced apoptosis. Cancer research. 2004; 64:6461–6468. [PubMed: 15374955]
16. Rao S, Porter DC, Chen X, Herliczek T, Lowe M, Keyomarsi K. Lovastatin-mediated G1 arrest is through inhibition of the proteasome, independent of hydroxymethyl glutaryl-CoA reductase. Proceedings of the National Academy of Sciences of the United States of America. 1999; 96:7797–7802. [PubMed: 10393901]
17. Trebicka J, Hennenberg M, Odenthal M, Shir K, Klein S, Granzow M, et al. Atorvastatin attenuates hepatic fibrosis in rats after bile duct ligation via decreased turnover of hepatic stellate cells. Journal of hepatology. 2010; 53:702–712. [PubMed: 20633948]
18. Miyaki T, Nojiri S, Shinkai N, Kusakabe A, Matsuura K, Iio E, et al. Pitavastatin inhibits hepatic steatosis and fibrosis in non-alcoholic steatohepatitis model rats. Hepatology research : the official journal of the Japan Society of Hepatology. 2011; 41:375–385. [PubMed: 21276150]
19. Marcelli M, Cunningham GR, Haidacher SJ, Padayatty SJ, Sturgis L, Kagan C, et al. Caspase-7 is activated during lovastatin-induced apoptosis of the prostate cancer cell line LNCaP. Cancer research. 1998; 58:76–83. [PubMed: 9426061]
20. Avins AL, Manos MM, Ackerson L, Zhao W, Murphy R, Levin TR, et al. Hepatic effects of lovastatin exposure in patients with liver disease: a retrospective cohort study. Drug safety : an international journal of medical toxicology and drug experience. 2008; 31:325–334.
21. Henderson LM, Patel S, Giordano TP, Green L, El-Serag HB. Statin therapy and serum transaminases among a cohort of HCV-infected veterans. Digestive diseases and sciences. 2010; 55:190–195. [PubMed: 19731022]
22. Lewis JH, Mortensen ME, Zweig S, Fusco MJ, Medoff JR, Belder R, et al. Efficacy and safety of high-dose pravastatin in hypercholesterolemic patients with well-compensated chronic liver disease: Results of a prospective, randomized, double-blind, placebo-controlled, multicenter trial. Hepatology. 2007; 46:1453–1463. [PubMed: 17668878]

23. Di Bisceglie AM, Shiffman ML, Everson GT, Lindsay KL, Everhart JE, Wright EC, et al. Prolonged therapy of advanced chronic hepatitis C with low-dose peginterferon. The New England journal of medicine. 2008; 359:2429–2441. [PubMed: 19052125]
24. Ishak K, Baptista A, Bianchi L, Callea F, De Groote J, Gudat F, et al. Histological grading and staging of chronic hepatitis. Journal of hepatology. 1995; 22:696–699. [PubMed: 7560864]
25. Ghany MG, Lok AS, Everhart JE, Everson GT, Lee WM, Curto TM, et al. Predicting clinical and histologic outcomes based on standard laboratory tests in advanced chronic hepatitis C. Gastroenterology. 2010; 138:136–146. [PubMed: 19766643]
26. Siegel AB. An ounce of prevention is better than a pound of cure: a patient-centered approach to hepatocellular carcinoma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2013; 31:1499–1501. [PubMed: 23509320]
27. Hernandez-Gea V, Friedman SL. Pathogenesis of liver fibrosis. Annual review of pathology. 2011; 6:425–456.
28. Mallat A, Preaux AM, Blazejewski S, Dhumeaux D, Rosenbaum J, Mavier P. Effect of simvastatin, an inhibitor of hydroxy-methylglutaryl coenzyme A reductase, on the growth of human Ito cells. Hepatology. 1994; 20:1589–1594. [PubMed: 7982659]
29. Atkins GB, Jain MK. Role of Kruppel-like transcription factors in endothelial biology. Circulation research. 2007; 100:1686–1695. [PubMed: 17585076]
30. Lin Z, Kumar A, SenBanerjee S, Staniszewski K, Parmar K, Vaughan DE, et al. Kruppel-like factor 2 (KLF2) regulates endothelial thrombotic function. Circulation research. 2005; 96:e48–57. [PubMed: 15718498]
31. SenBanerjee S, Lin Z, Atkins GB, Greif DM, Rao RM, Kumar A, et al. KLF2 Is a novel transcriptional regulator of endothelial proinflammatory activation. The Journal of experimental medicine. 2004; 199:1305–1315. [PubMed: 15136591]
32. Marrone G, Russo L, Rosado E, Hide D, Garcia-Cardena G, Garcia-Pagan JC, et al. The transcription factor KLF2 mediates hepatic endothelial protection and paracrine endothelial-stellate cell deactivation induced by statins. J Hepatol. 2013; 58:98–103. [PubMed: 22989565]
33. Tsan YT, Lee CH, Ho WC, Lin MH, Wang JD, Chen PC. Statins and the risk of hepatocellular carcinoma in patients with hepatitis C virus infection. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2013; 31:1514–1521. [PubMed: 23509319]
34. Yamaguchi A, Tazuma S, Nishioka T, Ohishi W, Hyogo H, Nomura S, et al. Hepatitis C virus core protein modulates fatty acid metabolism and thereby causes lipid accumulation in the liver. Digestive diseases and sciences. 2005; 50:1361–1371. [PubMed: 16047488]
35. Kapadia SB, Chisari FV. Hepatitis C virus RNA replication is regulated by host geranylgeranylation and fatty acids. Proceedings of the National Academy of Sciences of the United States of America. 2005; 102:2561–2566. [PubMed: 15699349]
36. Rao GA, Pandya PK. Statin therapy improves sustained virologic response among diabetic patients with chronic hepatitis C. Gastroenterology. 2011; 140:144–152. [PubMed: 20833169]
37. Delang L, Paeshuyse J, Vliegen I, Leyssen P, Obeid S, Durantel D, et al. Statins potentiate the in vitro anti-hepatitis C virus activity of selective hepatitis C virus inhibitors and delay or prevent resistance development. Hepatology. 2009; 50:6–16. [PubMed: 19437494]
38. Atsukawa M, Tsubota A, Kondo C, Itokawa N, Narahara Y, Nakatsuka K, et al. Combination of fluvastatin with pegylated interferon/ribavirin therapy reduces viral relapse in chronic hepatitis C infected with HCV genotype 1b. Journal of gastroenterology and hepatology. 2013; 28:51–56. [PubMed: 22989264]
39. Bader T, Fazili J, Madhoun M, Aston C, Hughes D, Rizvi S, et al. Fluvastatin inhibits hepatitis C replication in humans. The American journal of gastroenterology. 2008; 103:1383–1389. [PubMed: 18410471]
40. Kim SS, Peng LF, Lin W, Choe WH, Sakamoto N, Kato N, et al. A cell-based, high-throughput screen for small molecule regulators of hepatitis C virus replication. Gastroenterology. 2007; 132:311–320. [PubMed: 17241881]
41. Bader T, Hughes LD, Fazili J, Frost B, Dunnam M, Gonterman A, et al. A randomized controlled trial adding fluvastatin to peginterferon and ribavirin for naive genotype 1 hepatitis C patients. J Viral Hepat. 2013; 20:622–627. [PubMed: 23910646]

42. Kondo C, Atsukawa M, Tsubota A, Itokawa N, Fukuda T, Matsushita Y, et al. An open-label randomized controlled study of pegylated interferon/ribavirin combination therapy for chronic hepatitis C with versus without fluvastatin. J Viral Hepat. 2012; 19:615–622. [PubMed: 22863265]
43. Georgescu E, Catu D, Stoica RM, Ionescu RI. Fluvastatin as out-of-label enhancer for early (EVR) and sustained (SVR) virological response in chronic hepatitis C treated with peginterferon and ribavirin. J Hepatol. 2010; 52:S110.
44. Dimitroulakos J, Lorimer IA, Goss G. Strategies to enhance epidermal growth factor inhibition: targeting the mevalonate pathway. Clinical cancer research : an official journal of the American Association for Cancer Research. 2006; 12:4426s–4431s. [PubMed: 16857822]
45. Zhao TT, Le Francois BG, Goss G, Ding K, Bradbury PA, Dimitroulakos J. Lovastatin inhibits EGFR dimerization and AKT activation in squamous cell carcinoma cells: potential regulation by targeting rho proteins. Oncogene. 2010; 29:4682–4692. [PubMed: 20562912]
46. Lupberger J, Zeisel MB, Xiao F, Thumann C, Fofana I, Zona L, et al. EGFR and EphA2 are host factors for hepatitis C virus entry and possible targets for antiviral therapy. Nature medicine. 2011; 17:589–595.
47. Fuchs BC, Hoshida Y, Fujii T, Wei L, Yamada S, Lauwers GY, et al. EGFR inhibition attenuates liver fibrosis and development of hepatocellular carcinoma. Hepatology. 2013:n/a–n/a.
48. Zafra C, Abraldes JG, Turnes J, Berzigotti A, Fernandez M, Garca-Pagan JC, et al. Simvastatin enhances hepatic nitric oxide production and decreases the hepatic vascular tone in patients with cirrhosis. Gastroenterology. 2004; 126:749–755. [PubMed: 14988829]
49. Abraldes JG, Albillos A, Banares R, Turnes J, Gonzalez R, Garcia-Pagan JC, et al. Simvastatin lowers portal pressure in patients with cirrhosis and portal hypertension: a randomized controlled trial. Gastroenterology. 2009; 136:1651–1658. [PubMed: 19208350]

List of Abbreviations in the order of appearance

HCV	hepatitis C virus
CHC	chronic hepatitis C
HCC	hepatocellular carcinoma
HMG CoA	3-hydroxy-3-methylglutaryl-coenzyme A
HSCs	hepatic stellate cells
HALT-C	Hepatitis C Antiviral Long-term Treatment Against Cirrhosis
HAI	histology activity index
BMI	body mass index
ALT	alanine aminotransferase
AST	aspartate aminotransferase
HR	hazard ratio
CI	confidence interval
αSMA	α-smooth muscle actin
TGFβ	transforming growth factor-β
PDGF	platelet-derived growth factor
CTGF	connective tissue growth factor
KLF2	kruppel-like factor 2

SEC	sinusoidal endothelial cells
EGF	epidermal growth factor
EGFR	epidermal growth factor receptor

Table 1

Baseline clinical, laboratory and histological characteristics of patients with non-cirrhotic fibrosis (N=543), according to statin use.

Variable*	No Statin use (N=514)	Statin use (N=29)	*P*-value:
Age, years	50.1 (7.2)	54.2 (7.2)	0.002
Male, %	71.2	58.6	0.15
Race			0.02
• White, %	73.2	51.7	
• Black, %	18.9	44.8	
• Hispanic, %	4.9	3.5	
• Other, %	3.1	0.0	
Diabetes, %	16.9	58.6	<0.0001
Duration of infection, years	27.6 (8.0)	28.9 (8.8)	0.44
Alcohol history – median grams/day (IQR)	11.1 (2.0-29.6)	3.7 (0.0–25.4)	0.17
Past smoking history, %	73.2	75.9	0.83
BMI	29.5 (5.4)	30.0 (4.6)	0.63
HCV Genotype 1, %	94.2	100.0	0.30
Log_{10} HCV RNA (IU/mL)	6.5 (0.5)	6.5 (0.6)	0.66
ALT, U/L	110.9 (91.7)	68.1 (40.3)	<0.0001
AST, U/L	78.2 (53.3)	51.5 (21.5)	<0.0001
AST:ALT ratio	0.8 (0.3)	0.8 (0.2)	0.73
Total bilirubin, mg/dL	0.7 (0.4)	0.7 (0.3)	0.32
Albumin, g/dL	4.0 (0.3)	4.0 (0.3)	0.98
Alkaline Phosphatase, U/L	91.6 (42.6)	88.0 (24.5)	0.45
Platelets ×1000/mm^3	187.4 (60.2)	200.4 (53.1)	0.26
Ishak Fibrosis score	3.2 (0.6)	3.1 (0.6)	0.62
Ishak Fibrosis score			1.00
• 2, %	13.3	13.8	
• 3-4, %	86.7	86.2	
Histology Activity Index	7.3 (2.0)	7.2 (1.9)	0.94
Steatosis	1.3 (1.0)	1.3 (1.1)	1.00

Variable*	**No Statin use (N=514)**	**Statin use (N=29)**	***P*-value:**
Steatosis			0.20
• <1%	21.0	24.1	
• 1-5%	39.5	41.4	
• 6-33%	28.6	13.8	
• ≥34%	10.9	20.7	
Metformin use, %	10.3	48.2	<0.0001
Other lipid-lowering agent use[1], %	3.3	58.6	<0.0001
Randomization to treatment group, %	49.6	55.2	0.57

*Variables expressed as Mean (SD) unless indicated otherwise.

[1]Other lipid-lowering agents include: fibrates (clofibrate, fenofibrate, gemfibrozil), niacin, ezetimibe, bile acid sequestrants (cholestyramine, colesevelam, colestipol)

Table 2

Complementary log-log regression analysis of relationship between continuous statin use, and time to two-point increase in Ishak Fibrosis Score, among patients with baseline non-cirrhotic fibrosis (N=543).

		Unadjusted			Adjusted*		
Variable; N	**Fibrosis Progression N (%)**	**β coefficient**	**HR (95% CI)**	***P*-value**	**β coefficient**	**HR (95% CI)**	***P*-value**
Continuous Statin use; 29	3 (10%)	-1.15	0.32 (0.10–0.99)	0.048	-1.18	0.31 (0.10-0.97)	0.044
No Statin use; 514	145 (28%)		1.00			1.00	

*Hazard ratio adjusted for BMI, platelet count/50, (platelet count/50)^2 and hepatic steatosis score.

Table 3

Mean change in Ishak fibrosis score, HAI, and serum ALT level over the 3.5-year study observation period, by continuous statin use

Variable	Mean Change (SD)	*P*-value[1]
Ishak Fibrosis Score		
Statin Use (N=26)	- 0.34 (0.94)	0.006
No Statin Use (N=430)	+ 0.42 (1.42)	
HAI		
Statin Use (N=26)	- 1.27 (2.16)	0.15
No Statin Use (N=430)	- 0.52 (2.78)	
ALT, U/L		
Statin Use (N=29)	- 15.8 (40.5)	0.06
No Statin Use (N=461)	- 22.0 (93.2)	

[1] *P* values obtained using analysis of covariance adjusted for Ishak fibrosis score, HAI score and ALT at baseline.