Atorvastatin and Fluvastatin Are Associated With Dose-Dependent Reductions in Cirrhosis and Hepatocellular Carcinoma, Among Patients With Hepatitis C Virus: Results From ERCHIVES

Tracey G. Simon,1,2* Hector Bonilla,3* Peng Yan,4,5 Raymond T. Chung,1,2 and Adeel A. Butt4–6

Statins are associated with delayed fibrosis progression and a reduced risk of hepatocellular carcinoma (HCC) in chronic hepatitis C virus (HCV). Limited data exist regarding the most effective type and dose of statin in this population. We sought to determine the impact of statin type and dose upon fibrosis progression and HCC in patients with HCV. Using the Electronically Retrieved Cohort of HCV Infected Veterans (ERCHIVES) database, we identified all subjects initiated on HCV antibody (anti-HCV) therapy from 2001 to 2014, and all incident cases of cirrhosis and HCC. Statin use was measured using cumulative defined daily dose (cDDD). Multivariable Cox’s proportional hazard regression models were used to examine the relationship between statin use and development of cirrhosis and HCC. Among 9,135 eligible subjects, 1,649 developed cirrhosis and 239 developed incident HCC. Statin use was associated with a 44% reduction in development of cirrhosis (adjusted hazard ratio [HR]: 0.6; 95% confidence interval [CI]: 0.53, 0.68). The adjusted HRs (95% CI) of fibrosis progression with statin cDDD 28-89, 89-180, and >180 were 0.74 (0.59, 0.93), 0.71 (0.59, 0.88), and 0.6 (0.53, 0.68), respectively. Mean change in FIB-4 score with atorvastatin (n = 944) and fluvastatin (n = 34) was -0.17 and -0.13, respectively (P = 0.04), after adjustment for baseline FIB-4 score and established predictors of cirrhosis. Statin use was also associated with a 49% reduction in incident HCC (adjusted HR: 0.51; 95% CI: 0.36, 0.72). A similar dose-response relationship was observed. Conclusion: In patients with chronic HCV, statin use was associated with a dose-dependent reduction in incident cirrhosis and HCC. Atorvastatin and fluvastatin were associated with the most significant antifibrotic effects, compared with other statins. (HEPATOLOGY 2016;64:47-57)

SEE EDITORIAL ON PAGE 13

Hepatitis C virus (HCV) is one of the most common causes of chronic liver disease (CLD) and the leading indication for liver transplantation worldwide.1,2 Estimates suggest that over a period of 20–30 years, cirrhosis will develop in 10%-25% of patients with chronic hepatitis C (CHC) and hepatocellular carcinoma (HCC) in 1%-5%.2 Despite the great success of oral direct-acting antiviral

Abbreviations: ACE, angiotensin-converting enzyme; anti-HCV, HCV antibody; AVT, antiviral treatment; cDDD, cumulative defined daily dose; CHC, chronic hepatitis C; CI, confidence interval; CLD, chronic liver disease; DDD, defined daily dose; ERCHIVES, the Electronically Retrieved Cohort of HCV Infected Veterans; HBsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HDL, high-density lipoprotein; HF, hepatic fibrosis; HIV, human immunodeficiency virus; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; HR, hazard ratio; HSC, hepatic stellate cell; ICD-9, International Classification of Diseases, Ninth Revision (ICD-9); IQR, interquartile range; LDL, low-density lipoprotein; SD, standard deviation; SVR, sustained virological response; TC, total cholesterol; TG, triglyceride; VA, Department of Veterans Affairs.

Received October 19, 2015; accepted January 17, 2016.
Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1002/hep.28506/suppinfo.
Supported by NIH K24 DK078772 (to R.T.C.).
*These are co-first authors.
Copyright © 2016 by the American Association for the Study of Liver Diseases.
View this article online at wileyonlinelibrary.com.
DOI 10.1002/hep.28506
Potential conflict of interest: Dr. Butt has received investigator initiated grants (to the institution) from Gilead and AbbVie.
medications, there are still many patients with fibrosis and other clinical complications of HCV, who remain at risk of disease progression despite successful viral clearance. In such patients, reducing the risk of complications related to hepatic fibrosis, including cirrhosis and HCC, are of paramount importance.

3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, or statins, exert beneficial effects on circulating lipid levels and are used for management and prevention of coronary heart disease and stroke. Recently, statins have garnered attention for their pleiotropic effects as well. By both HMG-CoA-dependent and -independent pathways, statins have been shown to exert antiproliferative, antiangiogenic, proapoptotic, and immunomodulatory actions. They inhibit cell growth, decrease proteolysis, block tumor cell spread and may offer chemoprevention against many malignancies, including HCC.

It has been postulated that statins may also exert unique antifibrotic effects in HCV. In animal models, statins have been shown to block activation of hepatic myofibroblasts, inducing apoptosis and preventing proliferation of hepatic stellate cells (HSCs) and their production of collagens. In human studies, use of statins has been associated with a reduced risk of hepatic fibrosis (HF) progression and decreased incidence of HCC. In a recent analysis of 7,248 U.S. veterans infected with HCV, statin use was significantly associated with reduction in fibrosis progression rate and a lower adjusted risk of developing cirrhosis (hazard ratio [HR] = 0.56; 95% confidence interval [CI] = 0.50, 0.63) and HCC (HR = 0.51; 95% CI = 0.34, 0.76).

The potency of statin-mediated antiviral effects is thought to depend on the type of statin used, with fluvastatin having the most potent, and pravastatin the least potent, antiviral activity. However, data regarding the impact of statin type and dose upon hepatic fibrogenesis and risk of HCC remain limited. Recently, Yang et al. reported the results of a population-based cohort study of Taiwanese patients infected with HCV. The researchers observed a significant dose-dependent relationship between increasing statin dose and reduction in risk of cirrhosis. However, the subjects did not have antibody or viral load–confirmed diagnoses of HCV, nor did the study adjust for statin type, baseline disease activity, or fibrosis severity. We sought to assess the relationship between statin type and dosage with both HF progression and development of HCC in a large, well-established national cohort of HCV-infected U.S. veterans.

Patients and Methods

STUDY POPULATION

Study participants included patients with HCV infection within the Electronically Retrieved Cohort of HCV Infected Veterans (ERCHIVES). ERCHIVES, which has been described in multiple previous publications, is a large, established national cohort of HCV-infected veterans along with uninfected controls, created from multiple national databases. Briefly, all HCV-infected veterans observed at any of the nationwide Department of Veterans Affairs (VA) medical facilities who had a positive HCV antibody test between 2001 and 2014 were identified. Demographic, clinical, and laboratory data were obtained from the National Patient Care Database and the Corporate Data Warehouse, and pharmacy information, including all prescriptions written, doses, duration, number of pills, number of refills, and date of refills, was retrieved from the Pharmacy Benefits Management System.
Patients were included in the study cohort if they received at least 14 days of treatment for HCV. Patients were excluded if they were coinfected with human immunodeficiency virus (HIV), had a positive hepatitis B surface antigen (HBsAg), baseline cirrhosis, or HCC. Patients were also excluded if baseline HCV RNA or FIB-4 score were missing and if at least one FIB-4 score was not available at least 24 months after completion of HCV antibody (anti-HCV) therapy.

DEFINITIONS

Baseline was defined as the date of HCV treatment initiation. If multiple courses of treatment were given, the date of the most recent course was used for this analysis. Treatment completion was defined according to approved U.S. Food and Drug Administration labeling guidelines. Cirrhosis was defined as a FIB-4 score of >3.5 based upon previously published work by our group and others and was calculated as follows:

\[ \text{FIB-4} = \frac{\text{age} \times \text{AST [IU/L]} / \text{platelet count [platelets} \times 10^9 / \text{L}] \times (\text{ALT}^{1/2} [\text{IU/L}])}{\text{platelets}} \]

Baseline laboratory values were based on the results of testing performed closest to, but within 365 days preceding, the start date of antiviral therapy (AVT). Laboratory data were obtained at yearly intervals, and FIB-4 score was recalculated at each interval. An average of two values closest to the selected time point of interest was used for calculation of FIB-4 scores. Sustained virological response (SVR) was defined as undetectable HCV RNA in all follow-up HCV-RNA tests after the end of treatment, including at least one test more than 12 weeks after end of initial treatment.

Patients were defined as having diabetes if they satisfied at least one of the following: (1) International Classification of Diseases, Ninth Revision (ICD-9), coding for diabetes mellitus; (2) at least two outpatient random blood sugars greater than 200 mg/dL; or (3) were being prescribed antidiabetic medications before the start of AVT. History of alcohol and drug abuse or dependence and diagnosis of HCC were based on the presence of at least one inpatient or two outpatient ICD-9 diagnoses.

EXPOSURE TO STATIN MEDICATIONS

All patients who were prescribed statin medications in any VA pharmacy during the study observation period were identified. Statin prescriptions included simvastatin, lovastatin, fluvastatin, pravastatin, atorvastatin, and cerivastatin. We collected the dates of prescriptions ordered, the number of days prescribed, number of pills per prescription, and number of refills ordered. With this information, the statin defined daily dose (DDD) was calculated for each subject. The DDD is a validated unit for measuring a prescribed drug amount and is defined as the average maintenance dose per day of a drug consumed in an adult. It is calculated as:

\[ \text{DDD} = \frac{\text{total amount of drug prescribed on a daily basis to a patient}}{\text{amount of drug in a DDD}}. \]

The DDD was recalculated annually for each year of the study observation period.

The cumulative defined daily dose (cDDD) was calculated from the DDD. The cDDD is defined as the total sum of dispensed DDGs of a given medication. Both the DDD and cDDD are recommended by the World Health Organization and are widely used for comparison of medications, including statins, along a similar standard. Statin use was defined as >28 cDDDs of statin medications prescribed during the study period. Similar information was collected for nonstatin lipid-lowering agents (cholestyramine, colesuevam, colesterol, ezetimibe, niacin, and niacinamide), triglyceride (TG)-lowering agents (clofibrate, fenofibrate, and gemfibrozil), as well as the antidiabetic agents, Metformin, sulfonylureas, and thiazolidinedione.

OUTCOMES

Primary outcome measures were (1) progression of liver fibrosis as measured by FIB-4 score, (2) development of cirrhosis as defined by FIB-4 score ≥3.5, and (3) incident HCC, as described above.

STATISTICAL ANALYSIS

The study cohort was divided into statin users and nonusers, with statin use as defined above. Baseline demographic and clinical factors were compared between the two groups using chi-square test or t test, as appropriate. Predictors of incident cirrhosis and HCC were determined using Cox’s proportional

49
hazards analysis and generating HRs for each of the predictor variables. Mean change in fibrosis score over the study period was calculated for each of the statin medication by subtracting the baseline score from last available score. Mean fibrosis scores were also plotted over time by the cDDD of statins used. Kaplan-Meier’s curves were generated to demonstrate time to development of cirrhosis and HCC by statin cDDD. SAS (SAS Institute Inc., Cary, NC) and Stata software (version 11; Stata Corp LP, College Station, TX) were used for statistical analyses.

REGULATORY APPROVAL

This study was approved by the institutional review board at the VA Pittsburgh Healthcare System (Pittsburgh, PA). Appropriate permissions were obtained from the sources that provided data for ERCHIVES.

Results

BASELINE CLINICAL DATA

Within the ERCHIVES database, we identified 47,549 subjects with confirmed HCV infection, who were initiated on anti-HCV therapy during the study period. We excluded those who received both boceprevir and telaprevir (n = 32), and those with HIV coinfection (n = 766), as well as those who had positive HBsAg (n = 6,353), baseline cirrhosis (n = 2,867) or HCC (n = 941), or missing or undetectable HCV RNA at baseline (n = 3,571 and 2,235, respectively), or those without follow-up HCV RNA to measure SVR (n = 18,007). We also excluded those without sufficient baseline or follow-up labs to calculate FIB-4 scores (n = 1,301 and n = 2,341, respectively; Fig. 1).

Among the 9,135 remaining subjects, 4,165 (45.6%) were statin users and 4,970 (54.4%) were nonusers. A comparison of baseline demographic and clinical characteristics is shown in Table 1. Mean age was 53 among both statin users and nonusers (standard deviation [SD] 6.88 and 5.85, respectively; P < 0.0001). A similar percentage of both groups (65%) were white, and there was no significant difference in median HCV-RNA levels between groups. Median length of follow-up among statin users was 97.9 months (interquartile range [IQR]: 66.2, 126.6), compared to 81.6 months (IQR, 52.9, 113.6) among nonusers (P < 0.0001).

Statin users were more likely to be diabetic (24% vs. 9%; P < 0.0001) and to be prescribed metformin, other lipid-lowering medications, or angiotensin-converting enzyme (ACE) inhibitors (all P < 0.0001). Compared to nonusers, statin users had higher baseline total cholesterol (TC), low-density lipoprotein (LDL) and triglyceride (TG) levels, and lower high-density lipoprotein (HDL); all P < 0.0001. In bivariate analysis, patients who were prescribed statins were more likely to achieve SVR (55.1% vs. 47.5%; P < 0.0001), less likely to develop cirrhosis (14% vs. 21.4%; P < 0.0001), and less likely to be diagnosed with HCC (1.75% vs. 3.22%; P < 0.0001) than nonusers.

FIBROSIS PROGRESSION

Statin use was associated with reduced risk of fibrosis progression (HR, 0.66; 95% CI: 0.6, 0.73; P < 0.001). In multivariate Cox regression analysis, treatment with statins was associated with a significantly reduced risk of developing cirrhosis (adjusted HR: 0.64; 95% CI: 0.57, 0.72; P < 0.0001 after adjusting
Over 10 years of follow-up, statin use was also significantly correlated with a reduced rate of fibrosis progression compared to nonusers, at all doses ($P < 0.01$) and at all time points, with the exception of two $P$ values of 0.02 and 0.04 at two time intervals for the higher cDDD (Fig. 2).

There was also a significant dose-response relationship between statin use and reduction in fibrosis progression (Table 2). To further define this relationship,

**TABLE 1. Baseline Clinical, Laboratory, and Histological Characteristics of HCV-Infected Patients Without Baseline Cirrhosis (n = 9,135) According to Statin Medication Use**

| Variable† | No Statin Use (N = 4,970) | Statin Use† (N = 4,165) | P Value |
|-----------|---------------------------|-------------------------|---------|
| Age, years | 52.5 (6.88) | 53.5 (5.85) | <0.0001 |
| Male, % | 95.37 | 96.16 | 0.06 |
| Race, % | | | <0.0001 |
| White | 65.01 | 65.31 | |
| Black | 16.84 | 19.54 | |
| Hispanic | 5.77 | 5.35 | |
| Other | 12.37 | 9.80 | |
| Diabetes, % | 8.87 | 24.03 | <0.0001 |
| Alcohol abuse history, % | 38.79 | 34.81 | <0.0001 |
| Past smoking history, % | 90.44 | 92.65 | 0.0002 |
| BMI | 28.16 (5.79) | 29.57 (5.83) | <0.0001 |
| HCV genotype, % | | | <0.0001 |
| 1 | 31.65 | 25.55 | |
| 2 | 8.19 | 8.91 | |
| 3 | 5.84 | 4.66 | |
| 4 | 0.36 | 0.31 | |
| Mix | 0.12 | 0.07 | |
| Missing | 53.84 | 60.50 | |
| Log₁₀ HCV RNA (IU/mL) | 6.31 (1.95) | 6.17 (1.99) | 0.002 |
| ALT, U/L | 77.44 (64.56) | 73.73 (63.16) | 0.01 |
| AST, U/L | 54.62 (35.09) | 50.49 (32.94) | <0.0001 |
| Baseline lipid levels, mean (SD) | | | |
| TC | 163.54 (33.06) | 179.25 (39.08) | <0.0001 |
| LDL | 97.83 (29.49) | 110.41 (34.5) | <0.0001 |
| HDL | 42.74 (14.82) | 39.56 (13.14) | <0.0001 |
| TG | 123.85 (85.99) | 155.22 (106.72) | <0.0001 |
| Non-HDL cholesterol | 121.31 (31.67) | 139.81 (37.6) | <0.0001 |
| Platelets × 1,000/mm³ | 212.21 (62.93) | 217.02 (64.31) | 0.0003 |
| FIB-4 score | 1.7 (0.73) | 1.61 (0.69) | <0.0001 |
| Metformin use, % | 10.91 | 34.19 | <0.0001 |
| Other lipid-lowering agent use‡, % | 4.02 | 15.53 | <0.0001 |
| ACE inhibitor use, % | 38.11 | 65.7% | <0.0001 |
| Treatment regimen (most recent), % | | | <0.0001 |
| PEG/RBV only | 88.79 | 93.18 | |
| PEG/RBV/BOC | 10.08 | 6.36 | |
| PEG/RBV/TPV | 1.15 | 0.46 | |
| Completed course of HCV treatment§, % | 63.54 | 68.43 | <0.0001 |
| Attainment of SVR, % | 47.53 | 55.1 | <0.0001 |
| Incident cirrhosis (FIB-4 score >3.5) | 21.43 | 14.02 | <0.0001 |
| HCC | 3.22 | 1.75 | <0.0001 |
| Median length of follow-up, months (IQR) | 97.9 (66.2, 126.6) | 81.6 (52.9, 113.6) | <0.0001 |

*Values obtained at baseline or closest value obtained within 12 months of baseline. The exception was log₁₀ HCV RNA, for which the closest value to baseline that was obtained within 24 months of baseline was used.

†Variables expressed as mean (SD), unless indicated otherwise.

‡Other lipid-lowering agents include: fibrates (clofibrate, fenofibrate, and gemfibrozil), niacin, ezetimibe, and bile acid sequestrants (cholestyramine, colesvelam, and colestipol).

§A full course of treatment was defined as per the labeling guidelines for the particular regimen, taking into account previous treatment and presence of cirrhosis.

Statin use was defined as ≥28 cDDD per year; nonuse was defined as <28 cDDD per year.

Abbreviations: BMI, body mass index; ALT, alanine aminotransferase; AST, aspartate aminotransaminase; PEG, pegylated interferon; RBV, ribavirin; BOC, boceprevir; TPV, telaprevir.

for baseline FIB-4 score and univariate predictors of cirrhosis). Over 10 years of follow-up, statin use was also significantly correlated with a reduced rate of fibrosis progression compared to nonusers, at all doses ($P < 0.01$) and at all time points, with the exception of two $P$ values of 0.02 and 0.04 at two time intervals for the higher cDDD (Fig. 2).
Statin users were divided into three groups based upon yearly statin cDDD. Mean change in FIB-4 scores according to statin cDDD is shown in Supporting Fig. 1. For patients with cDDDs of 28-89, 90-180, and >180, the adjusted HR of fibrosis progression among statin users was 0.74 (95% CI: 0.59, 0.93; \( P = 0.01 \)), 0.71 (95% CI: 0.59-0.86; \( P = 0.0006 \)), and 0.6 (95% CI: 0.53, 0.68; \( P < 0.0001 \)), respectively, compared to nonusers (Table 2).

Mean change in FIB-4 score according to statin type is shown in Table 3. Overall, the greatest reduction in FIB-4 scores was observed with atorvastatin and fluvastatin, compared with other statins and compared with the change from baseline (mean change in FIB-4 score of -0.17 and -0.13, respectively; \( P = 0.04 \) after adjustment for baseline FIB-4 scores and other univariate predictors of cirrhosis).

DEVELOPMENT OF HCC

Statin use was also associated with a dose-dependent reduction in the incidence of HCC (Table 4). Those subjects who received more than 90 cDDDs demonstrated a significant reduction in HCC risk (\( P = 0.004 \)), and the greatest benefit was observed in those who received >180 cDDDs (\( P < 0.0001 \)). For subjects with 90-180 cDDDs and >180 cDDDs, adjusted HRs of incident HCC were 0.48 (95% CI: 0.27, 0.88; \( P = 0.02 \)) and 0.51 (95% CI: 0.36, 0.72; \( P = 0.0001 \)). Increasing statin dose was also associated with significantly delayed time to development of HCC (Fig. 2B).

In order to estimate whether the anti-HCC effects of statins were independent of their antiviral and antifibrotic effects, we conducted two sensitivity analyses

### TABLE 2. Complementary Log-Log Regression Analysis of Statin Use According to cDDD and Progression of Liver Fibrosis∗ Among Subjects With Baseline FIB-4 Score <3.5 (n = 9,135)

| Variable, (N) | Fibrosis Progression∥ | Unadjusted HR | Adjusted HR† |
|--------------|------------------------|---------------|--------------|
|              | N (%)                  | \( \beta \) Coefficient | HR (95% CI) | P-Value | \( \beta \) Coefficient | HR (95% CI) | P-Value |
| Statin use‡  | (4,165)                |                |              |          |                |              |          |
| cDDD 28-89 (543) | 81 (14.92)            | -0.44          | 0.65 (0.52, 0.81) | 0.0002    | -0.31          | 0.74 (0.59, 0.93) | 0.01    |
| cDDD 90-180 (815) | 122 (14.97)           | -0.42          | 0.66 (0.54, 0.79) | <0.0001   | -0.34          | 0.71 (0.59, 0.86) | 0.0006  |
| cDDD >180 (2,807) | 381 (13.57)          | -0.58          | 0.56 (0.43, 0.73) | <0.0001   | -0.51          | 0.6 (0.53, 0.68)  | <0.0001 |
| No statin use (4,970) | 1,065 (21.43)       | 1              | 1             |          | 1              | 1             |          |

*Progression of fibrosis: defined as any follow-up FIB-4 score \( \geq 3.5 \) during study observation period in patients with baseline FIB-4 score <3.5.

†Statin use defined as ≥28 cDDDs of statin medications, over study observation period.

‡HR adjusted for age, sex, race, smoking history, alcohol abuse history, body mass index, diabetes, baseline FIB-4 score, metformin use, ACE inhibitor use, other lipid-lowering agent use, past completed anti-HCV treatment, attainment of SVR, and daily caffeine intake.
of HCC risk. The first was stratified by mean change in FIB-4 score between baseline and year 1 of follow-up. We excluded those subjects who derived an antifibrotic benefit from statin use, defined as mean annual reduction in FIB-4 score ≥0.4, as has been validated in previously published literature.(34) This adjustment did not significantly impact the relationship between statin use and HCC risk (HR, 0.6 among patients who received >180 cDDDs; 95% CI: 0.38, 0.94; P = 0.03 after adjusting for baseline FIB-4). In a separate sensitivity analysis, we excluded subjects who had attained SVR, and this adjustment also did not diminish the strength of the association between statin cDDD and risk of either fibrosis progression or incident HCC.

**Discussion**

To our knowledge, this is the first U.S. study to demonstrate a dose-response relationship between statin use and reduction in HF progression and development of HCC after controlling for the potentially confounding effects of age, sex, HCV-RNA level, baseline level of fibrosis, alcohol use, smoking history, and concomitant use of potentially beneficial medications, such as metformin or other lipid-lowering agents. It is also the first study to document a relationship between statin type and fibrosis progression. In the final adjusted model, statin use was associated with a 44% overall reduction in risk of fibrosis progression and a 49% reduction in the incidence of HCC.

**FIBROSIS PROGRESSION**

When patients were stratified according to cumulative statin use, we observed a statistically significant inverse relationship between increasing statin dose and reduction in HR of fibrosis progression and HCC at

| Statin Type (N) | Mean Change (SD) | P Value\(^1\) |
|----------------|------------------|---------------|
| FIB-4 score    |                  |               |
| Atorvastatin (944) | -0.17 (1.01) | 0.04          |
| Fluvastatin (34) | -0.13 (0.91)  |               |
| Lovastatin (86)  | 0.4 (1.9)      |               |
| Pravastatin (609) | -0.03 (2.2)   |               |
| Rosuvastatin (187) | -0.07 (0.98) |               |
| Simvastatin (2,305) | 0.11 (4.92)  |               |
| No statin use (4,969) | 0.26 (2.12) |               |

Note: lipophilic statin medications included atorvastatin, lovastatin, and simvastatin. Hydrophilic statin medications included rosuvastatin, fluvastatin, and pravastatin.

\(^1\)Overall change defined as baseline FIB-4 score subtracted from the last FIB-4 score at end of study period.

**TABLE 4. HR of Statin Use and Reduction of HCC Risk Among Included Subjects (n = 9,135)**

| Model                                                                 | Nonusers*  | 28-89 cDDDs | 90-180 cDDDs | >180 cDDDs |
|-----------------------------------------------------------------------|------------|-------------|--------------|------------|
| HCC status, no. of patients                                          |            |             |              |            |
| • With                                                                | 160        | 12          | 12           | 49         |
| • Without                                                             | 4,810      | 531         | 803          | 2,758      |
| HCC status, no. of person-years                                      |            |             |              |            |
| • With                                                                | 863.06     | 65.84       | 54.16        | 299.43     |
| • Without                                                             | 33,556.21  | 3,936.89    | 5,957.27     | 225,288.5  |
| Incidence rate (per 10\(^6\) person-years from baseline)              | 464.86     | 299.8       | 199.62       | 214.65     |
| Absolute risk reduction\(^7\)                                        | n/a        | 166.06      | 268.24       | 250.21     |
|                                                                 | HR (95% CI) | P Value | HR (95% CI) | P Value | HR (95% CI) | P Value |
| Crude HR                                                              | 1.00       | 0.63 (0.35, 1.13) | 0.12 | 0.42 (0.23, 0.75) | 0.004 | 0.43 (0.31, 0.6) | <0.0001 |
| Adjusted HR\(^8\)                                                    | 1.00       | 0.85 (0.47, 1.53) | 0.58 | 0.48 (0.27, 0.88) | 0.02 | 0.51 (0.36, 0.72) | 0.0001 |

*Statin use defined as ≥28 cDDDs of statin medications over study observation period.

\(^7\)Absolute risk reduction per 10\(^6\) person-years from baseline.

\(^8\)HR adjusted for age, sex, race, smoking history, alcohol abuse history, caffeine intake, body mass index, diabetes, baseline FIB-4 score, metformin use, ACE inhibitor use, other lipid-lowering agent use, nonsteroidal anti-inflammatory medication use, past completed HCV treatment, attainment of SVR, and daily caffeine intake.

Abbreviation: n/a, not applicable.
each time point during the 10-year follow-up period. These associations were statin specific and remained significant even after adjustment for established predictors of both fibrosis progression and HCC. Therefore, these results add to the growing body of data showing that statins not only treat dyslipidemia, but also delay disease progression in patients with HCV.\(^{22,24}\)

Statins are among the most commonly prescribed medications worldwide and have been shown to offer significant benefit to patients with CLD\(^{11,22}\) through pleiotropic antiproliferative, -angiogenic, -inflammatory, and -neoplastic actions.\(^{6-8}\) Despite this, clinical data regarding the antifibrotic effects of statins in CHC remain limited. In a recent analysis of the HALT-C Trial cohort, a significant association between statin use and reduced fibrosis progression was observed,\(^{22}\) and similar results were found in a large national cohort study of U.S. veterans with CHC.\(^{24}\) However, until recently, the impact of dose, duration, or statin type upon HF progression had not been evaluated, and this is the first study to do so in a U.S. population.

Yang et al. recently reported a dose-response relationship between statin use and fibrosis progression in a population-based cohort from a national Taiwanese database.\(^{28}\) However, this study was limited in several important ways. First, diagnosis of HCV was defined only by reported ICD-9 code, rather than by HCV antibody or confirmatory HCV RNA. Similarly, a diagnosis of cirrhosis was defined only by ICD-9 code, without additional direct or surrogate clinical measurements. It is conceivable that cirrhosis—particularly compensated cirrhosis—may have gone unrecognized in a disproportionate group of patients, resulting in underestimation or misclassification of outcomes. Additionally, fibrosis severity, which is an important predictor of cirrhosis risk, was not assessed at baseline or at follow-up time points, and the study did not adjust for other potential confounders, including baseline HCV viral load, obesity, alcohol use, or smoking status.

A number of mechanisms have been proposed to explain the antifibrotic effects of statins, related to their antiviral and immunomodulatory effects.\(^{5}\) 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.\(^{35,36}\) They block the activation of hepatic myofibroblasts, inducing apoptosis and preventing HSC proliferation and collagen synthesis.\(^{6,7,13,19-21}\) They have also been shown to up-regulate transcription factors that exert vasoprotective effects in the liver and inhibit stellate cells.\(^{37}\) Finally, and perhaps most important, statins have demonstrated clinical efficacy in treatment of the metabolic syndrome, the components of which are independent risk factors for HCC among patients with CHC.\(^{38-40}\)

**STATIN TYPE AND FIBROSIS PROGRESSION**

Whether certain types of statins possess greater antifibrotic potential in CHC has been previously unknown. Our study is the first to compare the effects of different statin types on fibrosis progression in this population. We observed the greatest antifibrotic benefit with atorvastatin and fluvastatin, compared with simvastatin, pravastatin, lovastatin, or no statin use. This association remained significant even after adjustment for established predictors of cirrhosis. In clinical studies, fluvastatin has been associated with potent anti-HCV effects and enhanced rates of SVR\(^{26,41}\) and a recent analysis showed a reduction in nonalcoholic steatohepatitis-mediated fibrosis through inhibition of paracrine signaling with fluvastatin use.\(^{42}\) Future studies with large sample sizes and histological endpoints will be necessary in order to validate our findings.

**STATIN USE AND HCC**

This is the first U.S. study to demonstrate a significant dose-response relationship between statin use and reduction in the incidence of HCC. We observed a 47% overall reduction in incident HCC among statin users, which is in accord with previously published values, including a recent meta-analysis that reported a 37% reduced risk of HCC among statin users.\(^{38}\) As with the analyses of fibrosis, this dose-response relationship was statin specific and remained significant after adjustment for other established predictors of HCC. Moreover, in sensitivity analysis, the strength of this association was not diminished by exclusion of subjects who had attained SVR.

Statins may exert chemoprotective effects through inhibition of thioredoxin, a hepatic enzyme that is increased in premalignant hepatic nodules and plays a role in cell survival.\(^{43}\) By blocking cyclins and cyclin-dependent kinases,\(^{44}\) statins induce tumor cell apoptosis\(^{45}\) and microtubule bundling,\(^{46}\) while also promoting cell-cycle arrest.\(^{45}\) Statins also interfere with lipid rafts and inhibit tumor cell adhesion and
migration. In vitro, HCC cells treated with statins have been found to decrease expression of cell adhesion molecules, thus preventing cell growth and invasion possibly by a rho-dependent kinase.

It has also been postulated that the lipid-lowering action of statins may directly result in chemoprevention. Cancer cells undergo metabolic reprogramming that increases lipid biosynthesis and enhances expression of enzymes within the mevalonate pathway; this up-regulation has been associated with mutations in tumor-suppressor genes, increased cell spread, and development of HCC. Conversely, suppression of these pathways results in tumor suppression and apoptosis.

Strengths of this study include the use of a large, unselected national cohort of U.S. subjects, with long length of follow-up and serial measurements of clinical and laboratory parameters. Medication information was obtained from an integrated, comprehensive pharmacy database, which collected all prescription data, including dose, quantity, and refills. Additionally, to minimize confounding by indication and by severity of liver disease, we excluded patients with baseline cirrhosis, baseline HCC, and adjusted for baseline fibrosis scores in the final multivariable model. Though residual confounding cannot be completely excluded in an observational study, a dose-dependent relationship between statin use and reduced fibrosis progression and incident HCC was observed in all subjects, regardless of baseline severity of disease.

With a nonrandomized study design, this analysis was subject to potential selection bias and unmeasured confounding variables. However, with such a large cohort, we were able to control for many observed and well-described confounders in our adjusted models. A second potential limitation is use of a surrogate clinical score, rather than liver biopsy or transient elastography, for measurement and determination of HF stage. However, the FIB-4 score is a well-validated, widely used marker of liver fibrosis progression in the published literature. In addition, use of such a large sample size may minimize the variance otherwise attributable to this index score. Finally, it must be underscored that prescription data are inherently imprecise, and true patient adherence is often significantly reduced in comparison to prescription pharmacy information.

In conclusion, our findings demonstrate a significant dose-dependent reduction in risk of both fibrosis progression and incident HCC among statin users with CHC. These results support the possible role for statins in prevention of liver disease progression. Future prospective studies with histological and clinical endpoints are eagerly awaited. Such analyses will need to define the optimal timing of statin initiation, ideal duration of therapy, and relative antifibrotic potential of different types of statins in patients with CHC as well as other etiologies of liver disease.

Acknowledgment: This material is the result of work supported with resources and the use of facilities at the VA Pittsburgh Healthcare System and the central data repositories maintained by the VA Information Resource Center, including the National Patient Care Database, Decisions Support System Database, and Pharmacy Benefits Management Database. The views expressed in this article are those of the authors and do not necessarily reflect the position or policy of the Department of Veterans Affairs.

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. Ann Intern Med 2006;144:705-714.
2) Thomas DL, Seeff LB. Natural history of hepatitis C. Clin Liver Dis 2005;9:383-398, vi.
3) van der Meer AJ, Veldt BJ, Feld JJ, Wedemeyer H, Dufour JF, Lammert F, et al. Association between sustained virological response and all-cause mortality among patients with chronic hepatitis C and advanced hepatic fibrosis. JAMA 2012;308:2584-2593.
4) Stone NJ, Robinson JG, Lichtenstein AH, Bairey Merz CN, Blum CB, Eckel RH, et al. 2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. J Am Coll Cardiol 2014;63:2889-2934.
5) Sun HY, Singh N. Antimicrobial and immunomodulatory attributes of statins: relevance in solid-organ transplant recipients. Clin Infect Dis 2009;48:745-755.
6) 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 Res 2004;64:6461-6468.
7) Rao S, Porter DC, Chen X, Herlizcze T, Lowe M, Keyomarsi K. Lovastatin-mediated G1 arrest is through inhibition of the proteasome, independent of hydroxymethyl glutaryl-CoA reductase. Proc Natl Acad Sci U S A 1999;96:7797-7802.
8) Kuoppala J, Lamminpaa A, Pukkala E. Statins and cancer: a systematic review and meta-analysis. Eur J Cancer 2008;44:2122-2132.
9) Kalluri R, Neilson EG. Epithelial-mesenchymal transition and its implications for fibrosis. J Clin Invest 2003;112:1776-1784.

10) Kisseleva T, Brenner DA. Mechanisms of fibrogenesis. Exp Biol Med 2008;233:109-122.

11) Schuppan D, Afshari NH. Liver cirrhosis. Lancet 2008;371:838-851.

12) Iwaisako K, Brenner DA, Kisseleva T. What’s new in liver fibrosis? The origin of myofibroblasts in liver fibrosis. J Gastroenterol Hepatol 2012;27(Suppl 2):65-68.

13) Shirin H, Sharvit E, Aeed H, Gavish D, Bruck R. Atorvastatin and rosuvastatin do not prevent thioacetamide induced liver cirrhosis in rats. World J Gastroenterol 2013;19:241-248.

14) Kisseleva T, Brenner DA. Anti-fibrogenic strategies and the regression of fibrosis. Best Pract Res Clin Gastroenterol 2011;25:305-317.

15) Demierre MF, Higgins PD, Gruber SB, Hawk E, Lippman SM. Statins and cancer prevention. Nat Rev Cancer 2005;5:930-942.

16) Welzel TM, Graubard BI, Zeuzem S, El-Serag HB, Davila JA, McGlynn KA. Metabolic syndrome increases the risk of primary cancer in the United States: a study in the SEER-Medicare database. Hepatology 2011;54:463-471.

17) Bonovas S, Filoussi K, Tsavaris N, Sitaras NM. Statins and cancer risk: a literature-based meta-analysis and meta-regression analysis of 35 randomized controlled trials. J Clin Oncol 2006;24:4808-4817.

18) 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.

19) Trebicka J, Hennenberg M, Odenhal 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. J Hepatol 2010;53:702-712.

20) Miyaki T, Nojiri S, Shinoki N, Kusakabe A, Matsuura K, Iio E, et al. Pitavastatin inhibits hepatic steatosis and fibrosis in non-alcoholic steatohepatitis model rats. Hepatol Res 2011;41:375-385.

21) Marcelli M, Cunningham GR, Haidacher SJ, Padayatty SJ, Marzouk K, et al. Caspase-7 is activated during lovastatin-induced apoptosis of the prostate cancer cell line LNCaP. Cancer Res 1998;58:76-83.

22) Simon TG, King LY, Zheng H, Chung RT. Statin use is associated with a reduced risk of fibrosis progression in chronic hepatitis C. J Hepatol 2015;62:18-23.

23) 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. J Clin Oncol 2013;31:1514-1521.

24) Butt AA, Yan P, Bonilla H, Abou-Samra AB, Shaikh OS, Simon TG, et al. Effect of addition of statins to antiviral therapy in hepatitis C virus-infected persons: results from ARCHIVES. Hepatology 2015;62:362-374.

25) Ikeda M, Abe K, Yamada M, Dansako H, Naka K, Kato N. Different anti-HCV profiles of statins and their potential for combination therapy with interferon. Hepatol Res 2006;39:117-125.

26) Bader T, Fazili J, Madhoun M, Aslam A, Monnier MF, et al. Matrix conditions and KLF2-dependent induction of heme oxygenase-1 modulate inhibition of HCV replication by fluvastatin. PLoS One 2014;9:e96353.

27) Yang YH, Chen WC, Tsan YT, Chen MJ, Shih WT, Tsai YH, Chen PC. Statin use and the risk of cirrhosis development in patients with hepatitis C virus infection. J Hepatol 2015;63:1111-1117.

28) Butt AA, Yan P, Lo Re V 3rd, Rimensdorfer D, Goetz MB, Leaf D, et al. Liver fibrosis progression in hepatitis C virus infection after seroconversion. JAMA Intern Med 2015;175:178-185.

29) Department of Veterans Affairs Hepatitis C Resource Center, Yee HS, Currie SL, Darling JM, Wright TL. Management and treatment of hepatitis C viral infection: recommendations from the Department of Veterans Affairs Hepatitis C Resource Center program and the National Hepatitis C Program office. Am J Gastroenterol 2006;101:2360-2378.

30) Backus LL, Boothroyd DB, Phillips BR, Mole LA. Predictors of response of US veterans to treatment for the hepatitis C virus. Hepatology 2007;46:37-47.

31) Zeuzem S, Heathcote EJ, Shiffman ML, Wright TL, Bain VG, Sherman M, et al. Twelve weeks of follow-up is sufficient for the determination of sustained virologic response in patients treated with interferon alpha for chronic hepatitis C. J Hepatol 2003;39:106-111.

32) World Health Organization. World Health Organization Collaborating Center for Drugs Statistical Methodology: ATC Index with Defined Daily Dose. Oslo, Norway: World Health Organization (WHO); 2003.

33) Tamaki N, Kurozaki M, Tanaka K, Suzuki Y, Hoshioka Y, Kato T, et al. Noninvasive estimation of fibrosis progression over time using the Fib-4 index in chronic hepatitis C. J Viral Hepat 2013;20:72-76.

34) Kapadia SB, Chisari VF. Hepatitis C virus RNA replication is regulated by host geranylgeranylation and fatty acids. Proc Natl Acad Sci U S A 2005;102:2561-2566.

35) Clendening JW, Penn LZ. Targeting tumor cell metabolism with statins. Oncogene 2012;31:4967-4978.

36) Marrone G, Maeso-Diaz R, Garcia-Cardenas G, Abraldes JG, Garcia-Pagan JC, Bosch J, et al. KL2F exerts antifibrotic and vasoprotective effects in cirrhotic rat livers: behind the molecular mechanisms of statins. Gut 2015;64:1433-1444.

37) Siegel AB, Zhi AX. Metabolic syndrome and hepatocellular carcinoma: two growing epidemics with a potential link. Cancer 2009;115:5651-5661.

38) Ohki T, Tateishi R, Sato T, Masuizaki R, Imanura J, Goto T, et al. Obesity is an independent risk factor for hepatocellular carcinoma development in chronic hepatitis C patients. Clin Gastroenterol Hepatol 2008;6:459-464.

39) Konishi I, Hiai Y, Shigematsu S, Hirooka M, Furukawa S, Abe M, et al. Diabetes pattern on the 75 g oral glucose tolerance test is a risk factor for hepatocellular carcinoma in patients with hepatitis C virus. Liver Int 2009;29:1194-1201.

40) 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 naïve genotype 1 hepatitis C patients. J Viral Hepat 2013;20:622-627.

41) Chong LW, Hsu YC, Lee TF, Lin Y, Chiu YT, Yang KC, et al. Fluvastatin attenuates hepatic steatosis-induced fibrogenesis in rats through inhibiting paracrine effect of hepatocyte on hepatic stellate cells. BMC Gastroenterol 2015;15:22-22.

42) Skogastier CA, Johansson M, Parini P, Eriksson M, Eriksson LC, Ekstrom L, et al. Statins inhibit expression of thioredoxin reductase 1 in rat and human liver and reduce tumour development. Biochem Biophys Res Commun 2012;417:1046-1051.

43) Rejal B, Meder F, Wilhelm K, Henrich D, Marzi I, Lehner M. Simvastatin inhibits cell growth and induces apoptosis and G0/G1 cell cycle arrest in hepatic cancer cells. Int J Mol Med 2010;26:735-741.
45) Zhang W, Wu J, Zhou L, Xie HY, Zheng SS. Fluvastatin, a lipophilic statin, induces apoptosis in human hepatocellular carcinoma cells through mitochondria-operated pathway. Indian J Exp Biol 2010;48:1167-1174.

46) Ali N, Allam H, Bader T, May R, Basalingappa KM, Berry WL, et al. Fluvastatin interferes with hepatitis C virus replication via microtubule bundling and a doublecortin-like kinase-mediated mechanism. PLoS One 2013;8:e80304.

47) Relja B, Meder F, Wang M, Blaheta R, Henrich D, Marzi I, et al. Simvastatin modulates the adhesion and growth of hepatocellular carcinoma cells via decrease of integrin expression and ROCK. Int J Oncol 2011;38:879-885.

48) Farwell WR, Scranton RE, Lawler EV, Lew RA, Brophy MT, Fiore LD, et al. The association between statins and cancer incidence in a veterans population. J Natl Cancer Inst 2008;100:134-139.

49) Poynter JN, Gruber SB, Higgins PD, Almog R, Bonner JD, Rennert HS, et al. Statins and the risk of colorectal cancer. N Engl J Med 2005;352:2184-2192.

50) Hirsch HA, Iliopoulos D, Joshi A, Zhang Y, Jaeger SA, Bulyk M, et al. A transcriptional signature and common gene networks link cancer with lipid metabolism and diverse human diseases. Cancer Cell 2010;17:348-361.

51) Freed-Pastor WA, Prives C. Mutant p53: one name, many proteins. Genes Dev 2012;26:1268-1286.

52) Karlic H, Thaler R, Gerner C, Grunt T, Proestling K, Haider F, et al. Inhibition of the mevalonate pathway affects epigenetic regulation in cancer cells. Cancer Genet 2015;208:241-252.

Author names in bold designate shared co-first authorship.

Supporting Information

Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1002/hep.28506/suppinfo.