Rationale for the use of statins in liver disease

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Abbreviations

AngII, angiotensin II; BDL, bile duct ligation; CRP, c-reactive protein; CVD, cardiovascular disease; ECM, extracellular matrix; eNOS, endothelial nitric oxide synthase; EPC, endothelial progenitor cells; FPP, farnesyl-pyro phosphate; GGPP, geranylgeranyl-pyrophosphate; HBV, viral hepatitis B; HCC, hepatocarcinoma; HCV, viral hepatitis C; Hh, hedgehog; HMG-CoA, 3-hydroxy-3-methyl-glutaryl-coenzyme A; HSC, hepatic stellate cell; KLF2, kruppel-like factor 2; MHC-II, major histocompatibility complex class II; MMP, matrix metalloproteinase; MS, metabolic syndrome; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; NOX2, nicotinamide adenine dinucleotide phosphate oxidase isoform 2; PBC, primary biliary cirrhosis; PNPLA3, patatin-like phospholipase domain-containing protein 3; PPARα, activated peroxisome proliferator-activated receptor alpha; PSC, primary sclerosing cholangitis; SREBP, sterol regulatory element binding proteins; TACE, transarterial chemoembolization; UDCA, ursodeoxycholic acid.
Abstract

The evolution of chronic liver injuries from benign and manageable dysfunction to life-threatening end stage liver disease with severe complications renders chronic liver disease a global health burden. Due to the lack of effective medication, transplantation remains the only and final curative option for end stage liver disease. Since the demand for organ transplants by far exceeds the supply, other treatment options are urgently required to prevent progression and improve end stage liver disease.

Statins are primarily cholesterol-lowering drugs used for primary or secondary prevention of cardiovascular diseases. In addition to the primary effect, statins act beneficially through different pleiotropic mechanisms on inflammation, fibrosis, endothelial function, thrombosis and coagulation to improve chronic liver diseases. However, concerns remain about the efficacy and safety of statin treatment due to their potential hepatotoxic risks and as of now, these risks impede broader use of statins in the treatment of chronic liver diseases.

The aim of this review is to comprehensively describe the mechanisms by which statins improve prospects for different chronic liver diseases with special focus on the pathophysiological rationale and the clinical experience of statin use in the treatment of liver diseases.
**General underlying mechanisms of statins**

Statins are a class of competitive inhibitors of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme for endogenous cholesterol and isoprenoid synthesis in the mevalonate pathway. Its inhibition leads to decreased production of precursors and subsequently reduction of cholesterol biosynthesis (Figure 1A).

The so-called pleiotropic effects are mediated by the reduction of isoprenoids, such as farnesyl-pyrophosphate (FPP) and geranylgeranyl-pyrophosphate (GGPP), necessary for the activation of small GTPases like Rho- and Ras-proteins (Figure 1A). Statins diversely inhibit RhoA and Rac1 prenylation, modulating endothelial nitric oxide synthase (eNOS), NO availability and enhancing stability of eNOS mRNA (Figure 1B). This statin-dependent eNOS restoration can be also mediated by increased kruppel-like factor 2 (KLF2) expression (28). Similar effects were observed in hepatic injury followed by fibrosis and cirrhosis. Moreover, statins decrease Angiotensin II (AngII)-induced oxidative stress in the liver (34) and enhance eNOS expression and activity by inhibiting RhoA membrane association (2, 52). Taken together, inhibition of GTPase prenylation, restoration of eNOS and NO availability are the key roles of statins in the improvement of vascular and endothelial function (Figure 1B).

But also the decreased cholesterol synthesis itself is beneficial several fold. Decreased cholesterol level leads to an increase of sterol regulatory element binding proteins (SREBP), which act as transcription factors for the LDL receptor that induces higher plasma LDL clearance due to an increased LDL receptor-mediated uptake and after lysosomal degradation of LDL (17). Also, the decreased hepatic triglyceride synthesis is possibly associated with activated peroxisome proliferator-activated receptor α (PPARα) and increased β-oxidation activity (17) (Figure 1C).
Moreover, statins seem to beneficially modify PPARγ activity, which attenuates the production of tumor necrosis factor α (TNFα), interleukins 1-beta (IL1β) and 6 (IL6) and C-reactive protein (CRP) (19, 46).

In summary, due to various mechanisms, dependent or not on the cholesterol levels, they modify pathological conditions as outlined in the following.
Statins in cardiovascular diseases

Statins also decrease low-density lipoprotein (LDL) cholesterol levels, which are pro-
atherogenic. For this reason statins have become standard of care in the treatment of
diabetes and cardiovascular diseases (CVD) to decrease or even reverse
atherosclerosis. However, in liver diseases they are often underused even in high-
risk patients (9). Especially in CVD, several studies have shown a clear effect of
statins on inflammation. Besides the older Pravastatin Inflammation/CRP Evaluation
(PRINCE) study, also a recent study demonstrated that statins improve inflammation
and decrease interleukin-6 (IL-6) levels, a main regulator of CRP (19). Besides
cytokine expression, statins exert anti-inflammatory properties in endothelial cells
through modulation of activity and expression of metalloproteinases (MMPs) and
adhesion molecules. Furthermore, statins directly inhibit the expression of major
histocompatibility complex class II (MHC-II) molecules by interferon g (IFN-g) in
CD4+ helper T cells (TH1 cells), leading to a shift towards anti-inflammatory TH2 cell
actions and beneficially modifying atherosclerosis (25). Moreover, as outlined above
statins improve eNOS and NO in endothelium, which decreases leukocytes
chemotaxis, adhesion and inflammation, key mechanisms aggravating
atherosclerotic (25). The decreased infiltration of plaques by macrophages and
downregulation of proteolytic enzymes are associated also with decreasing NADPH
oxidase isoform 2 (NOX2) (40).

While statins might inhibit the immune cells activity they increase the number of
circulating endothelial progenitor cells (EPC), which are important in the
neovascularization of ischemic tissue, thereby contributing to the restoration of
endothelial function (36). Furthermore, statins elicits anti-thrombotic effects down-
regulating platelet CD40L, by inhibiting tissue factor activity and thrombin generation
(25, 17). The modulation of inflammation, matrix remodeling, thrombosis and
vascular function improves prognosis of CVD patients treated with statins.
Adverse effects and hepatotoxicity

Although similar mechanisms as in CVD are involved in development and progression of liver disease, caution is required due to hepatotoxicity. Hepatic cells make a considerable contribution to cholesterol production and therefore are a major target of statins. The pharmacological activity and the hepatic metabolism of statins depend on their molecular structure and physical properties such as lipophilicity, solubility and absorption. Simvastatin, lovastatin, fluvastatin, and atorvastatin are metabolized by cytochrome P450, while pravastatin, rosuvastatin, and pitavastatin remain almost unaffected by any hepatic metabolic processes.

The effect of statins on aminotransferase levels in the treatment of cardiovascular diseases was investigated in several studies with contradictory results. This inconsistency may be explained by pharmacogenetics and differences in the statins used. Although statins are generally well tolerated, reports about statin-induced liver injury can be found mainly for atorvastatin and simvastatin. However, this might be coincidental since these two statins are also the two most commonly prescribed ones (8).

The question whether statins have a hepatotoxic effect is considerably more relevant in patients with acute or chronic liver dysfunction. In a retrospective cohort study, lovastatin showed no increased risk of adverse hepatic effects in a total of 93,106 patients with liver disease. Another prospective randomized, double blind, placebo-controlled multicenter trial, investigated the safety of high-dose pravastatin in chronic liver disease. After 36 weeks of treatment, alanine aminotransferase (ALT) levels were even lower in the pravastatin treated group (26). Furthermore, HMG-CoA reductase inhibitors were also found to be safe in patients after liver transplantation (29).

Few studies revealed that statins hepatotoxicity is a rare condition and might mimic
an autoimmune phenotype of liver injury (4, 44, 45). However, in patients with chronic kidney diseases the incidence of severe adverse events seem to be higher (38). Additionally, in most cases the benefits of statins outweigh any potential hepatotoxic risks (8). Thus, comparing benefits and risks of statin treatment individually could help to improve its efficacy.
Distinct and common mechanisms of statins in liver diseases

CVD in many patients are associated with metabolic syndrome (MS), which in turn represents the ground of the development of NAFLD and NASH. Statins have been considered for treatment in NASH, and in recent years they have been generally evaluated as safe - even at high doses – leading to a wider use in patients (11, 26, 39). Nevertheless, studies assessing the beneficial effects of statins are scarce, mostly investigating only a small number of patients with different endpoints. These studies showed that statin therapy either attenuates inflammation and steatosis (15) or trending toward decreased fibrosis while other studies found no change in fibrosis (15, 35). The different study outcomes may be due to the different statins used in the respective trials. While atorvastatin at 10mg/d for 24 months elicited positive effects in NASH, simvastatin 20mg/d over 12 months had no effect in a similar cohort of patients (35). Moreover, genetic predispositions were disregarded by most of these studies and may provide additional explanation for the different outcomes regarding fibrosis. For example, a large multi-center study revealed that statin use in high risk patients is beneficial, except in patients carrying the PNPLA3 I148M risk alleles (14). Thus, genetic screening may be advisable for NAFLD patients to ascertain the optimal therapy for each patient.

Recent studies revealed improvements of NASH and MS after statin treatment (14, 16) (Table 1). Statins decrease LDL cholesterol levels in serum, and as a result, oxidized LDL levels which plays an important role in NASH. As highlighted in Figure 1C, statin therapy leads to decreased hepatic steatosis by decreasing LDL and activating SREBPs, PPARα and β-oxidation (17). However, the anti-inflammatory effect of statins in NAFLD and NASH is partly attributed to activation of PPARγ and subsequent downregulation of pro-inflammatory mediators (19, 46). Additionally, the inhibition of small GTPase prenylation and diminished downstream signaling...
contributing to the anti-inflammatory features of statins (46). Recent experimental NASH studies further suggest a beneficial impact of statins in fibrosis. They inhibit the paracrine signaling of hepatocytes on hepatic stellate cells (HSC), thereby inhibiting hepatic stellate cells (HSC) activation and fibrogenesis during experimental NASH (12).

Additionally to the hepatic and general metabolic improvement which are known to be tightly linked to chronic viral hepatitis C (HCV) infection, statins might exert direct anti-replicative effect on HCV (21). Previous small scale studies investigated the effect of statin monotherapy and revealed only mild antiviral effects (6). Especially in combination with direct-acting antiviral agents statins may have an added value to the antiviral efficacy and mitigate the progression of HCV-related diseases, such as cirrhosis or HCC (23, 37, 47, 57). Additionally, HCV patients with compensated cirrhosis under statin treatment seem to have a lower risk for decompensation as well as lower mortality (33) (Table 1).

Cholesterol synthesis and especially excretion might have an effect by default on biliary disorders. While increased cholesterol secretion contributed to gallstones formation, with possible complications such as cholangitis, cholecystitis or biliary pancreatitis, chronic inflammatory processes, in cholestasis, secretion of cholesterol into the bile is reduced, which increase cholesterol serum levels and might further influence bile acid production and thereby lead to hepatic damage especially in primary biliary cholangitis (PBC) (10). Thus, both disease complexes may be influenced by statins by decreasing biliary cholesterol secretion and serum cholesterol levels (Table 1). The antilithogenic effect of statins results from decreased biliary cholesterol secretion, reduced cholesterol saturation index and thus, reduced bile lithogenicity. Older studies suggest some additional benefits of statins in addition to ursodeoxycholic acid (UDCA) to prevent the development, but
not to treat established gallstones, and no novel insights have been established lately in the field. By contrast, statins reduce serum bile acids levels in bile duct ligated (BDL) mice (13), at least partly due to activation of pregnane X receptor/sterol x receptor and PPARα. Moreover, statins sufficiently reduce serum LDL levels and cholesterol and may also improve vascular function in PBC patients, without hepatic improvement, which might be also due to low numbers of patients (10, 50, 51). Therefore, further investigations should be performed to assess possible protective statin effects in PBC patients.

Either NASH, HCV, PBC or other etiology of liver disease have a common end-stage, characterized by a profound liver remodeling and portal hypertension. Portal hypertension in cirrhosis arises by a mechanically increased intrahepatic resistance by narrowing of hepatic outflow (55). An additional dynamic component of increased intrahepatic resistance is dominated by an imbalance of vascular tone-regulating pathways, showing a shift towards vasocontraction. Furthermore, RhoA and Rho-kinase signaling is responsible for the increased tone of the hepatic vasculature contributing to the activation of HSC (58), the major contributor to ECM synthesis upon chronic liver injury (18, 30). Statins modulate the mechanic and the dynamic intrahepatic pathways. Both pathways represent targets of statin therapy in liver cirrhosis with portal hypertension (Figure 1B). Atorvastatin inhibits the translocation of RhoA and thus the activity of Rho-kinase (29). This effect decreases collagen production and hepatic stellate cell activation in early fibrosis as well as proliferation, cytokine production and contraction of activated hepatic stellate cells in cirrhosis (52, 53). Importantly, statins might induce senescence in activated HSC leading to a decreased turnover of these highly active cells (24, 52, 53). Simultaneously, statins improve endothelial dysfunction by upregulated activity of eNOS and NO availability in cirrhotic livers and further decrease portal pressure (2, 52). Statin-induced
upregulation of eNOS is mediated by KLF2 to decreased HSC activation and lower portal pressure (27, 28) (Table 1).

In several small scale human studies, acute and chronic effects of simvastatin on portal pressure were investigated (1, 41). All studies show a significantly decreased hepatic vascular resistance in cirrhosis with portal hypertension, which was additional to the extrahepatic effect of beta-blockers (1).

Another recent study confirmed the beneficial effects of statins, even in patients identified as non-responder to non-selective beta-blockers (5). Besides decreased portal pressure, cirrhotic patients under statins may also benefit from improved liver function. Interestingly, statin effects are enhanced with increased severity of portal hypertension (41). In addition, simvastatin improved survival in patients after variceal hemorrhage while no effect was observed on the rebleeding rate, suggesting multiple beneficial effects of statins (3). As demonstrated in an animal model (31), the severity of hemorrhage might be also lower in patients receiving statins. Moreover, the toxicity of statins was less observed as a liver damage, but mainly as myopathy (3), which might be prevented and attenuated by novel statin drugs, as suggested by using a compound containing atorvastatin and a NO-donor (43).

Portal hypertension might result also from obstruction of the liver venous inflow or outflow (54). In non-cirrhotic portal hypertension, contrary to its effects in cirrhotic portal hypertension, statins might enhance splanchnic vascular hyperperfusion due to increased angiogenesis in non-cirrhotic portal hypertension mediated by an upregulation of the Hedgehog (Hh) pathway (56). Although cirrhotic and non-cirrhotic portal hypertension share endpoints, the origin of portal hypertension and the grade of liver disease possibly have a crucial impact on the treatment success. Hence, this study suggests caution when using statins in non-cirrhotic portal hypertension.

As mentioned before, especially in HCV cirrhosis, statins might exert anti-malignant
properties. Besides inhibition of proliferation *in vitro*, statins might also induce apoptosis of hepatoma cells and inhibit intrahepatic angiogenesis (Table 1). Reduced proliferation might be due to interferences with K-ras and prevention of p21 and 27 breakdown in malignant cells followed by induction of cell-cycle arrest. Specific interference with integrins and Rho-kinase, which are expressed at the cell membrane, were shown to reduce proliferation as well as tumor cell adhesion in an *in vitro* HCC model (42). These effects are mainly attributed to reduced RhoA activation and subsequently Rho-kinase inhibition (Figure 1). Recent studies report a highly significant decrease in HCC risk resulting from statin use (48, 49). The recently published data from the Electronically Retrieved Cohort of HCV Infected Veterans (ERCHIVES) database shows a dose-dependent reduction of fibrosis progression as well as HCC incidence. Remarkably, reduction of HCC incidence was about 47% in all treated patients. Also, this study clearly showed differences in statin efficacy, whereby atorvastatin and fluvastatin had the strongest effects on fibrosis progression, as well as HCC incidence (48). Nonetheless, it remains uncertain whether these effects are due to fibrosis reduction, direct effects on HCC progression or a combination of both. It should also be taken into account that statins might have antiviral properties due to reduction of HCV replication, additionally contributing to benefits observed in the ERCHIVES patients (6). Therefore, future studies are required to further evaluate statin therapy in the prevention of HCC.

Besides prevention of HCC incidence, pravastatin attenuates progression and metastatic behavior of HCC due to decreased metalloproteinase activity. Additionally, statins hamper Ras signaling and also decrease cholesterol production. The cholesterol content of mitochondrial membrane, which may be crucial in the development of chemoresistance in HCC, is also reduced by statins (Table 1). The data in this context are limited and controversial. While recently published findings
analyzing early stage HCC patients showed no significant difference in survival related to statin treatment (20), the additional effect of statins on chemotherapy as well as transarterial chemoembolization (TACE) treatment could be beneficial in HCC patients (22).
Summary/Conclusion

Statins exhibit pleiotropic effects in many liver diseases. One of these effects is the inhibition of isoprenoid synthesis as a consequence of decreased HMG-CoA reductase activity since the resulting modulated GTPase activity plays a major role in the treatment of most chronic liver diseases (Table 1).

Statins are cost-effective, generally well tolerated by patients and the benefits of statin treatment in most patients outweigh their potential hepatotoxic risk. Especially in patients with severe chronic liver injury and high risk of CVD, statin treatment is very promising since it not only prevents the development of CVD but also could help to prevent the progression of liver fibrosis to cirrhosis and HCC with all the concomitant complications. Therefore, reasons for statins use in chronic liver diseases are more convincing than reasons against, rendering statin treatment a definite advantage.
References


Legends to figures and tables:

Figure 1. Pleiotropic effects of statins on small GTPases. Statins interfere with cholesterol synthesis by inhibition of HMG-CoA reductase. The subsequent lack of mevalonate and sequential components impedes prenylation of small GTPases and thereby their activity (A). Generic downstream pathways by which RhoA affects contractility (B). The possible effects of decreased cholesterol synthesis (C).

Table 1. Statin effects in liver diseases. Statins could not only improve different liver diseases but may also prevent the development of cirrhosis and its complications.
A

Ras \(\rightarrow\) active

Ras \(\rightarrow\) inactive

Farnesyl-PP

Geranyl-PP

STATINS

HMG-CoA reductase

Acetyl-CoA

RhoA

Geranylgeranyl-PP

Squalene

B

RhoA

eNOS mRNA

Akt

Myosin light chain phosphatase

Myosin light chains

Relaxation

Contraction

C

Cholesterol \(\downarrow\)

LDL-Receptor \(\uparrow\)

SREBPs

PPAR\(\alpha\) \(\rightarrow\) \(\beta\)-oxidation \(\downarrow\)

PPAR\(\gamma\) \(\downarrow\)

Inflammation \(\downarrow\)
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| NAFLD/NASH                            | decrease LDL serum levels  
decrease hepatic inflammation  
inhibit HSC activation  
decrease hepatic fibrosis | 11, 12, 14, 15, 16, 17, 19, 26, 28, 35, 39, 46 |
| Chronic viral hepatitis C             | inhibit viral replication  
increase LDL uptake                                                              | 6, 21, 23, 33, 37, 47, 57 |
| Hepatobiliary diseases                | decrease cholesterol secretion  
decrease formation of cholesterol gallstones  
ameliorate cholestasis | 10, 13, 50, 51      |
| Liver fibrosis and portal hypertension| inhibit HSC activation  
decrease collagen production  
decrease portal pressure  
improve endothelial dysfunction  
induce senescence of HSC       | 1, 2, 3, 5, 24, 27, 28, 29, 31, 41, 43, 58, 52, 53 |
| Non-cirrhotic portal hypertension     | increase angiogenesis                                                          | 54, 56              |
| Hepatocellular carcinoma              | inhibit proliferation  
induce apoptosis  
inhibit angiogenesis  
decrease HCC incidence       | 6, 20, 22, 42, 48, 49 |