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Lipid modifiers and NASH: statins, ezetimibe, fibrates and other agents

RUNNING HEAD: lipid-lowering agents in NAFLD

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Abstract

Nonalcoholic fatty liver disease (NAFLD) frequently coexists with hyperlipidemia and conveys an increased cardiovascular risk, regardless of traditional risk factors. Furthermore, altered fatty acid and cholesterol metabolism are increasingly recognized to be central for the pathogenesis of liver injury in non-alcoholic steatohepatitis (NASH). Therefore, lipid-lowering drugs are attractive therapeutic tools in NAFLD.

Statins ameliorated surrogate markers of steatosis in several randomized controlled trials, but their impact on liver histology is unknown. Furthermore, statins were the only class of lipid-lowering drugs to reduced cardiovascular risk in NAFLD.

Preliminary evidence suggests ezetimibe, an inhibitor of intestinal and hepatic cholesterol absorption, may improve liver histology, but its impact on CVD risk and on clinical outcomes remains to be determined.

Despite strong experimental evidence supporting the use of omega-3 polyunsaturated fatty acids in NAFLD, the published human studies consisted of small sample size and had many methodological flaws, including the absence of post-treatment histology.

Key words: *NAFLD, lipid-lowering drugs, cholesterol-lowering drugs, hyperlipidemia, ezetimibe, statins, PUFA*

Key points

-NAFLD is associated with an increased cardiovascular disease (CVD) risk, regardless of associated traditional risk factors and of fasting plasma lipid values

-altered lipoprotein metabolism, cytokine balance and postprandial fat tolerance are believed to account for at least part of the increased CVD risk of NAFLD patients; there is also emerging evidence connecting hepatic free cholesterol accumulation to liver injury in NASH.

-in the light of increasing evidence connecting increased hepatic free cholesterol content to liver disease in NAFLD, cholesterol-lowering agents are receiving much attention for the treatment of NAFLD

-among statins, atorvastatin improved biochemical/radiological markers of steatosis in NAFLD, and reduced CVD events in the GREACE study. The benefit of statins on CVD risk seem to be even greater in patients with NAFLD than in patients with normal liver enzymes. The impact of statins on liver histology is unknown.

- in the few trials available, ezetimibe improved liver histology, but its glucose-related safety needs further evaluation

-these drugs may offer synergistic benefit when used in combination with each other or with other drugs targeting different steps of lipid/glucose metabolism.

Multiple choice questions (correct answer underlined)

1) Which are the putative mechanisms underlying the increased cardiovascular risk in NAFLD, independently of traditional risk factors

- a. altered lipoprotein metabolism, characterized by increased large triglyceride-rich lipoproteins, reduced HDL-cholesterol
- b. altered lipoprotein metabolism, characterized by increased large triglyceride-rich lipoproteins, reduced HDL-cholesterol, increased small LDLs
- c. altered lipoprotein metabolism, characterized by increased large triglyceride-rich lipoproteins, reduced HDL-cholesterol, increased small LDLs, increased oxidized LDLs
- d. altered pro/anti-inflammatory adipokine balance
- e. c+d

2) Which is currently the only drug that reduced the risk of incident CVD events in randomized controlled trials?

- a. simvastatin
- b. rosuvastatin
- c. statins as a class overall
- d. ezetimibe
- e. polyunsaturated fatty acids

3) What are the mechanisms whereby ezetimibe might benefit NAFLD?

- a. reduced intestinal cholesterol absorption
- b. reduced hepatic cholesterol uptake
- c. reduction in both intestinal cholesterol absorption and hepatic cholesterol uptake
- d. reduced stellate cell activation by intracellular cholesterol accumulation
- e. reduced adipokine secretion by adipose tissue

Introduction

Nonalcoholic fatty liver disease (NAFLD) can be encountered in up to 30% of the general adult population. It is considered the hepatic manifestation of the metabolic syndrome, affecting up to 60-80% of diabetic and obese patients [1]. NAFLD encompasses a histological spectrum ranging from simple steatosis (SS) to steatosis plus necro-inflammation (non-alcoholic steatohepatitis, NASH), with or without fibrosis, that can only be differentiated by liver biopsy; NASH affects 3-5% of the general adult population and up to 20-40% of obese and diabetic subjects [1].

Patients with NAFLD have an increased risk not only of liver-related complications (largely confined to those with NASH), but also of cardiovascular disease (CVD) and of type 2 diabetes. In a recent meta-analysis, the risk of incident and/or fatal CVD was 1.5-2-fold higher than the general population, respectively[2]. Importantly, the higher risk of CVD persisted even after adjusting for metabolic syndrome, suggesting NAFLD increases cardiovascular risk independently of associated risk factors[2]. Furthermore, CVD risk was increased in both SS and NASH cases, suggesting a reduction in cardiovascular risk should be pursued in all NAFLD patients, regardless of liver histology and of the presence of coexisting hyperlipidemia.

Mechanisms underlying this association are under investigation: even in the absence of overt dyslipidaemia (hypercholesterolemia or atherogenic dyslipidemia, i.e. elevated triacylglycerol (TG) and/or low HDL-cholesterol levels), fatty liver is constantly associated with more subtle alterations in lipoprotein subfractions and metabolism. This is because liver TG excess drives hepatic large very low density lipoprotein(VLDL)-1 subfraction oversecretion, which accumulate in the blood. These compete with chylomicrons for lipoprotein lipase and exchange their TG with LDL and HDL particles, eventually leading to the highly atherogenic phenotype of small, dense LDLs, and low HDL-C (prevalently represented by HDL-3) [3]. Furthermore, even in the presence of normal fasting lipid values, NAFLD is associated with reduced tolerance to dietary fat, characterized by increased postprandial lipemia, enhanced pro-inflammatory response, and altered postprandial lipoprotein metabolism and adipokine profile following fat ingestion[4]. Lastly, dysregulated fatty acid and cholesterol metabolism are increasingly recognized to play a crucial pathogenic role in liver injury in

NASH, as discussed below. Collectively, these considerations make lipid-lowering drugs an attractive therapeutic measure, even in normolipidemic NAFLD patients.

Cholesterol-lowering drugs

Growing animal and human evidence connects altered hepatocyte cholesterol metabolism and hepatic free cholesterol accumulation to the pathogenesis of liver injury in NASH. Mari et al first showed mitochondrial free cholesterol accumulation sensitizes the liver to inflammatory stimuli in rats through mitochondrial glutathione depletion [5]. Subsequently, other authors have demonstrated that cholesterol intake is essential for determining hepatic inflammation and NASH in hyperlipidemic mouse models fed a Western diet, likely through direct Kupffer cell activation upon scavenging of remnant cholesterol-rich lipoproteins; consistently, omitting cholesterol from the high-fat diet prevented hepatic free cholesterol accumulation, hepatocyte injury or apoptosis, macrophage recruitment, and the development of diet-induced NASH[6, 7]. Recent animal and human studies elegantly elucidated the mechanisms underlying hepatocyte free cholesterol accumulation: up-regulation of cholesterol synthesis and of low density lipoprotein receptors (LDLR) via activation of sterol regulatory element binding protein-2 (SREBP-2), reduced biotransformation to bile acids, and suppression of canalicular pathways for cholesterol and bile acid excretion in bile [7, 8].

In a parallel way, epidemiological studies have linked excessive dietary cholesterol intake to the development and severity of NAFLD, independently of caloric and macronutrient intake, even in the absence of overt hypercholesterolemia [9, 10], while lipidomic analysis of human livers has shown a progressive increase of hepatic free cholesterol during different stages of NASH [11, 12].

Altogether, these features make cholesterol-lowering drugs a potentially effective therapeutic approach in NAFLD/NASH [13, 14, 15, 16, 17, 18, 19, 20, 21] (Table 1).

Statins

The antioxidant and anti-inflammatory properties of HMG C0-A reductase inhibitors (“statins”), the frequent coexistence of NAFLD and dyslipidemia and the increased cardiovascular risk of these patients make statins an attractive therapeutic tool in NAFLD. Despite this premise, data on statin efficacy in NAFLD are sparse, at least in part due to their feared hepatotoxicity in subjects with coexisting liver disease. The fear for statins hepatotoxicity has been overcome a recent Liver Expert Panel statement that “patients with NAFLD are not at increased risk of statin hepatotoxicity and routine transaminase monitoring is not warranted in these subjects” [22]. Even their feared potential for worsening glucose tolerance seems largely outweighed by their well-established cardiovascular benefit [23].

To date, safety and efficacy of statins in NAFLD have been evaluated in 5 randomized controlled trials (RCTs) (Table 1). Two small RCT with post-treatment histology evaluated simvastatin and atorvastatin in NAFLD, finding either no change or an improvement in steatosis [13, 16]. While the impact of statins on liver histology and liver-related outcomes is largely unknown, emerging evidence suggests these drugs may benefit liver-unrelated clinical outcomes in NAFLD patients. For example, the Greek Atorvastatin and Coronary Heart Disease Evaluation (GREACE) study was a 3-year prospective, randomised, open label survival study enrolling 1600 patients (78% male; 20% diabetic; 45% with metabolic syndrome) with established coronary heart disease (CHD), serum LDL cholesterol >2.6 mmol/L and triglyceride <4.5 mmol/L; patients were randomized to receive statins or usual care (lifestyle changes, such as a low-fat diet, weight loss, and exercise, and all necessary drug treatment, including statin) [14]. The primary end-point was the first occurrence of any cardiovascular event. In a *post-hoc* analysis of the GREACE study, safety and efficacy of statins were evaluated in 437 patients (80% male; 51% diabetic; 91% with metabolic syndrome) with abnormal (< 3 x ULN) baseline transaminases, presumably due to NAFLD, based on ultrasonography and exclusion of other common causes of abnormal liver function tests. The risk reduction in NAFLD patients was compared with the relative risk reduction observed between patients with normal liver tests who were treated with a statin and those not treated with a statin.

Overall, statins (mainly atorvastatin 24 mg/d) were safe, with rate of discontinuation <1%

because of liver-related adverse effects (transaminase elevation >three-times the ULN). Importantly, diabetes incidence was low (4%) in statin-treated NAFLD patients and similar to that of patients not on statin (4.3%). Statins improved liver function tests in NAFLD, normalizing AST, ALT and GGT in 89% of patients after 3 years, while liver tests worsened in NAFLD patients not receiving statins. Beside a reduction in LDL-C (-44% from baseline), statin-treated NAFLD patients improved also plasma triglyceride (-32%), HDL-C (+8%) and estimated glomerular filtration rate (EGFR) (from 59 ml/min to 70 ml/min/1.73 m², p<0.0001). Most importantly, statin treatment was associated with a significant (-68%) risk reduction of CVD events compared with both NAFLD patients not on statin and with statin-treated patients with normal transaminases.

With all the limitation of a post-hoc analysis and the definition of NAFLD adopted by these investigators, some considerations can be drawn from this important study:

1) statins appear safe in NAFLD and actually improve biochemical markers of liver disease (raised serum transaminase). The actual steatosis regression rate with statins remains however unknown, as a substantial number of NAFLD patients still have significant liver fat infiltration despite normal liver enzymes.

1) the GREACE is the first RCT showing therapeutic benefit on clinical end-points in NAFLD. Statin-related CVD risk reduction was greater in patients with presumed NAFLD than in those with normal liver tests, with a number needed to treat of 5 NAFLD patients to save 1 CVD event. Therefore, the risk-to-benefit ratio of long-term treatment with statins (mainly atorvastatin in this study) seems to favour statin administration for patients with NAFLD-related moderately abnormal liver tests.

2) consistently with the results of a recent meta-analysis, statins are safe and do not seem to affect the risk of incident diabetes[23], although their impact on insulin resistance and glucose metabolism in NAFLD requires further evaluation.

3) the benefit of statins in NAFLD appear to extend to renal function. Emerging evidence connects NAFLD, and more consistently NASH, to an increased risk of developing chronic kidney disease, defined as either an estimated glomerular filtration rate of <60 mL/min/1.73 m² of body surface area or

abnormal proteinuria, independently of traditional risk factors, metabolic syndrome, and insulin resistance[24, 25, 26]. Mechanisms potentially linking obesity, NAFLD and kidney disease include altered fetuin-A, adiponectin and tissue 5'-AMP activated protein kinase (AMPK) expression [27] . Statins may benefit renal function through still unclear mechanisms, including transcriptional regulation of organic anion transporters (OATs) localized in the basolateral side of proximal renal tubular cells, like SLCO4C1, which are involved in the renal elimination of uremic toxins and kidney inflammation[28, 29].

3) atorvastatin was the most widely used statin in the GREACE study. It is currently unknown whether different statins have different effectiveness in NAFLD: atorvastatin was assessed in all but 1 RCT in NAFLD and constantly improved biochemical and radiological surrogates of steatosis, while simvastatin did not change liver histology in the only RCT with post-treatment histology [13]. These differences may be related to the varying lipophilicity and related extrahepatic effects of different statins, which need to be further addressed by future research[30] (Table 2; Table 3).

4) the median daily dose of statins (atorvastatin 24 mg, simvastatin 22 mg, pravastatin 31 mg, and fluvastatin 40 mg) in NAFLD patients who normalized liver tests was similar to that used in patients who did not normalize liver enzymes at the end of the study. Of the measured variables, only reduction in plasma triglyceride correlated with ALT improvement in the GREACE population ($r=0.59$, $p=0.002$). Therefore, a major issue for future RCTs should be the individuation of predictors of response to statin in NAFLD. Patients with NAFLD are a heterogeneous population, with different mechanisms contributing to liver injury in the single patient. A major challenge for research will be to individuate those genetic or environmental factors associated with a favourable response to statin treatment, to tailor treatment to individual patient characteristics.

Combination therapy with statins

As NASH may well be a heterogeneous disease (ie a similar tissue response to more than one interacting pathogenic factors), several studies evaluated statins in combination with other drugs

targeting different factors believed to be involved in the pathogenesis of NAFLD, including oxidative stress and renin-angiotensin system.

Foster et al conducted a post hoc analysis of the St. Francis Heart Study; they examined the effect of combined therapy with atorvastatin (20 mg/d), vitamin C (1 g/d), and vitamin E (1,000 IU/d) or placebo on 80 subjects with hepatic steatosis, assessed as the liver to spleen ratio from computed tomography[15]. The combination of atorvastatin with vitamins E and C significantly reduced the presence of NAFLD after 3.6 years (70% vs 34%; OR: 0.29; 95% CI, 0.15–0.79; $P < .001$). The improvement in hepatic steatosis was associated with significant reduction in the total and LDL cholesterol levels. However, HDL cholesterol and TG levels did not differ significantly between the two groups. The St. Francis RCT was originally designed to evaluate the effect of this treatment on the risk of developing CVD in healthy individuals deemed high risk by coronary calcium score. Several observations can be made on this study.

1) The exclusion of individuals with baseline transaminases $> 1.5 \times$ ULN likely excluded patients with moderate-to-severe NASH; furthermore, alcohol intake was not assessed, and some participants might actually have had alcoholic rather than non-alcoholic fatty liver.

2) Most importantly, it is unclear whether the post-treatment 2.2-fold reduction in NAFLD prevalence in the treatment arm compared to placebo was the effect of the combination of atorvastatin plus antioxidants or of either agent alone, given that vitamin E showed significant benefit on liver disease in the 2 large “Pioglitazone versus vitamin E for the treatment of non-alcoholic steatohepatitis” (PIVENS) and “Treatment of nonalcoholic fatty liver disease in children” (TONIC) trials [31, 32].

3) Significant reduction in NAFLD prevalence in the treatment group was still observed in patients with normal cholesterol and triglyceride levels at baseline and after controlling for age, sex, baseline BMI and blood pressure, and follow-up lipid values, suggesting that hyperlipidemia is not required for this treatment to be effective. Although the antioxidant effect of vitamins C and E may have contributed to the observed improvement, the documented antioxidant, anti-lipogenic, anti-inflammatory and antifibrogenic effects of statins may also have played a relevant role (Table 2)[16, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42]. Consistent with this view, in a small RCT enrolling 29 normocholesterolemic overweight subjects, atorvastatin 80 mg/d reduced liver TG content by 40%

compared with placebo. This effect was coupled with pervasidiverse?[or persuasive? Or multiple] changes in hepatic expression of genes involved in lipogenesis, including a reduction in the master nuclear transcription factor sterol regulatory element binding protein (SREBP)-1c and in angiopoietin-like protein 3 (ANGPTL3), while fasting plasma glucose, NEFA and insulin sensitivity were unchanged [14] (Table 1).

In another RCT on normocholesterolemic, nondiabetic hypertensive NAFLD patients, the combination of losartan plus simvastatin significantly improved steatosis as assessed by ultrasonography, abdominal visceral adiposity, insulin sensitivity, adiponectin and c-reactive protein compared to amlodipine plus simvastatin, despite similar blood pressure reduction[17]. This study further confirms statins are effective in NAFLD even in the absence of overt dyslipidemia, and that may synergize with drugs targeting other key pro-inflammatory pathways involved in the pathogenesis of liver disease and cardio-metabolic risk of NAFLD.

Ezetimibe

Ezetimibe inhibits the Niemann-Pick C1 like 1 (NPC1L1) protein, which catalyzes absorption of cholesterol by enterocytes and hepatocytes in the gut but also the biliary canaliculi of the liver. Use of ezetimibe in animal models of NASH has produced promising results [43, 44] .

In 2 nonrandomized trials, ezetimibe improved histological steatosis and necroinflammatory grade, but not fibrosis in NAFLD [18,19]. These improvements were associated with an improvement in insulin resistance and with a favourable redistribution in plasma VLDL/LDL lipoprotein subfractions, in unsaturated/saturated fatty acid profile and in estimated desaturase activity index [45].

Following these encouraging results, two RCTs assessed ezetimibe in NAFLD. In abdominally obese NAFLD patients, ezetimibe plus a low-fat diet significantly reduced magnetic resonance (MR)-assessed liver fat content and plasma inflammatory markers compared to placebo, despite similar weight, waist and HOMA reduction [20] (Table 1). In the first RCT with post-treatment histology,

ezetimibe significantly improved histological ballooning and fibrosis, but worsened HbA1c, suggesting the impact of this agent on glucose metabolism needs further evaluation [21].

As with statins, researchers evaluated the potential synergism of combination therapy of ezetimibe with other agents in NAFLD. To further reduce hepatotoxic free cholesterol accumulation, inhibition of cholesterol absorption by ezetimibe and synthesis by statins may offer additive benefits over either agent alone: this hypothesis has been currently tested in a small uncontrolled trial, where ezetimibe/simvastatin (10/20mg/d) significantly improved liver enzymes and atherogenic dyslipidemia in 19 diabetic patients with NAFLD [46].

The combination of ezetimibe with alpha-glucosidase inhibitors significantly improved liver histology compared with either drug alone, an effect at least in part explained by synergistic effects on secretion of the intestinal incretin, glucagon-like peptide-1, and on hepatic microsomal triglyceride transfer protein (MTP) and peroxisome proliferators-activated receptor- α 1 (PPAR- α 1) expression [47, 48, 49].

Consistent with these experimental findings, Nagai et al. reported dramatic improvement in liver enzymes and insulin resistance with ezetimibe+voglibose in a single? patient with NASH, refractory to ezetimibe+ursodeoxycholic acid [50].

N-3 polyunsaturated fatty acids (PUFA)

N-3 PUFA experimentally ameliorated hepatic steatosis by enhancing PPAR- α -mediated fatty acid catabolism and suppressing SREBP-1c-mediated *de novo* lipogenesis[51]. Furthermore, these agents possess anti-inflammatory and insulin-sensitizing properties, and also improved liver injury in animal models of NASH[52].

In a recent review, Musso et al critically appraised the published literature related to omega-3 fatty acids in NAFLD. They found that despite strong experimental evidence supporting their use in NAFLD, the published human studies consisted of small sample size and had many methodological flaws[53]

In 5 RCTs (303 participants), PUFA improved transaminase levels, plasma triglyceride and insulin resistance, and reversed radiological steatosis in 20-64% of cases[54, 55, 56, 57, 58] (Table 1). The impact of PUFA on liver histology is largely unknown: in the only RCT with post-treatment histology, PUFA ameliorated steatosis without affecting other histological features[58]. Overall, PUFA were well-tolerated, with minor gastrointestinal symptoms.

The considerable heterogeneity of study populations, treatment duration, doses, implementation of lifestyle intervention and of methods for assessing radiological steatosis prevents any definitive conclusion on these agents; further well-designed, adequately powered RCTs with histological endpoints are warranted.

Probucol

Probucol is a lipophilic lipid-lowering agent with strong antioxidant activity. It was evaluated in NASH in one well-designed RCT: transaminases improved, but post-treatment histology was unavailable [59] (Table 1). Although generally well-tolerated, probucol was associated with a significant fall in HDL-C, raising concerns about cardiovascular safety.

Fibrates

Fibrates are PPAR- α agonists that showed consistent anti-steatogenic activity in animal models of NAFLD[60]. In 5 RCTs (315 participants), fibrates improved plasma lipids, but had no effect on radiological/histological features of NAFLD and on glucose metabolism[14, 61, 62, 63, 64] (Table 1).

Conclusions

NAFLD is often associated with cardiovascular risk factors, including fasting and/or postprandial atherogenic dyslipidemia, characterized by higher triglyceride levels, increased small, dense LDL particles, and decreased HDL cholesterol, which may at least in part underly the increased CVD risk of these patients. Therefore, aggressive treatment of dyslipidemia should be considered in the overall framework of cardiovascular risk reduction in patients with NAFLD and associated dyslipidemia.

Furthermore, emerging evidence strengthens the use of lipid-lowering drugs even in NAFLD patients with normal fasting plasma lipids, as their CVD risk seems associated with more subtle alterations in lipoprotein metabolism, which are not caught by routine biochemical tests and are reversed by lipid-lowering drugs. In view of the global risk assessment of NAFLD patients, the NCEP ATP IV guidelines for the management of dyslipidemia are expected to be published in 2012, and they will likely provide state-of-the-art criteria for risk stratification and therapeutic targets. In the meantime, patients with NAFLD should be risk stratified and managed according to their cardiovascular risk, and their cardiovascular risk factors should be managed based on their risk status[65].

Statins are the first line agents to treat high cholesterol: among all statins, there is more experience with atorvastatin, which is the only statin to date to show a reduction in the incidence of CVD events in patients with NAFLD. Recent consensus panels have definitively stated the risk for liver injury from statins is quite rare and patients with NAFLD are not an increased risk for statin hepatotoxicity. Omega-3 fatty acids are perhaps the first choice to treat hypertriglyceridemia because of their safety, tolerability, and efficacy in improving serum triglycerides as well as their potential to improve the liver disease. Importantly, the effects of all lipid-lowering drugs on liver histology, the most widely accepted surrogate for liver-related risk, are unknown and need to be evaluated in future, adequately powered, well designed RCTs.

Concerning future research, if free cholesterol overload is confirmed as a key pathogenic factor for the development of NASH in humans, it will be important to evaluate the most effective strategy to unload liver cells of free cholesterol accumulation, which could be the simultaneous inhibition of cholesterol synthesis and canalicular uptake by statins plus ezetimibe or simultaneous modulation of hepatic cholesterol synthesis, uptake and excretion by targeting key nuclear transcription factors like SREBP-2.

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