

NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD): THE SEARCH FOR A CURE

Alabagi Abdulla, Charity Reynolds, *H Hesham A-Kader

*Division of Gastroenterology, Hepatology and Nutrition, Department of Pediatrics,
University of Arizona, Tucson, Arizona, USA*

**Correspondence to heshamh@aol.com*

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ABSTRACT

An alarming rise of obesity and, along with it, non-alcoholic fatty liver disease (NAFLD), has been observed in the USA and the rest of the world. NAFLD, the most common cause of chronic liver disease in many developed countries, is not always a benign disorder and considering its growing nature, will have a serious impact on healthcare systems worldwide. The search continues for a suitable therapy for this disorder; the therapy ideally needs to be safe, effective, and affordable. The biggest hurdle in the process of developing such a therapy is our lack of a complete understanding of the pathogenesis of the disease.

Keywords: Fatty liver, non-alcoholic fatty liver disease (NAFLD), steatosis, steatohepatitis, obesity.

INTRODUCTION

The problem of obesity has grown tremendously in the USA and the rest of the world and, along with it, several comorbidities including non-alcoholic fatty liver disease (NAFLD). NAFLD represents over 75% of chronic liver disease cases.¹ It is also one of the most common indications for liver transplantation, surpassed only by hepatitis C virus infection and alcoholic liver disease.² In the USA National Health and Nutrition Examination Survey, 6% of overweight and 10% of obese adolescents had an elevated serum alanine aminotransferase (ALT).³ Studies from Japan^{4,5} and England⁶ reported that the prevalence of the disease has almost doubled over the last two decades and a significant increase of its occurrence in adolescents has been observed (174%).^{1,7}

Although NAFLD was first described in 1980, we still lack a complete understanding of the mechanism and causes of the disease.⁸ There is a pressing need for the development of non-invasive diagnostic modalities, simple screening markers, and safe, effective, and affordable therapeutic agents. The aim of this article is to discuss the current, emerging, and potential therapeutic options for the management of NAFLD.

PATHOGENESIS AND NATURAL HISTORY

The pathogenesis of non-alcoholic steatohepatitis (NASH) is commonly described by the 'two-hit hypothesis': the first hit results from disorders of hepatic uptake, synthesis, degradation, and secretion of free fatty acids, which result in macrovesicular steatosis. This predisposes the liver to a second hit with progression from simple steatosis to steatohepatitis and cirrhosis.⁹

Fat-derived factors such as fatty acids, adiponectin, and tumour necrosis factor alpha (TNF α) modulate the hepatic inflammatory response, regulate the inflammatory response, and promote NAFLD. Adiponectin and TNF α are mutually antagonistic. Adiponectin hinders fatty acid uptake, incites fatty acid oxidation and the export of lipids, and increases hepatic insulin sensitivity. On the other hand, TNF α recruits inflammatory cells and promotes insulin resistance. The imbalance seen in patients with metabolic syndrome of increased production of TNF α associated with decreased activity of adiponectin enhances insulin resistance, resulting in fat deposition, inflammation, and cell death.^{10,11}

The natural history of patients with NAFLD is poorly defined. A large cohort study has shown that the hazard ratio for general mortality and liver-related

mortality was 1.038 and 9.32, respectively.¹² The leading cause of death in patients with NAFLD is cardiovascular disease.¹² Data regarding the progression of NAFLD from simple steatosis to steatohepatitis is conflicting. In a study that included 40 patients who were followed over a median period of 11 years, there was no progression to NASH or cirrhosis, and only 30% of the patients had abnormal liver tests at the conclusion of the study.¹³ Conflicting data was reported recently from a study of 52 NAFLD patients followed for 3 years. 15% had normal histology, 23% remained at baseline, 39% developed borderline NASH, and 23% developed NASH. Twenty-two of the 52 subjects had borderline NASH when the study commenced. At the conclusion of the study, 18% of the 22 patients had simple steatosis, 59% remained at borderline NASH, and 23% had NASH.¹⁴

The overall risk of a patient with simple steatosis progressing to cirrhosis is about 1–2%.¹⁵ On the other hand, the risk of cirrhosis in patients with NASH varies from 0% at 5 years to 12% at 8 years.^{16,17} Ekstedt et al.¹⁸ followed 129 patients for a mean of 13.7 years and reported that 5.4% of the patients developed end-stage liver disease, including hepatocellular carcinoma (HCC). In another study of 46 patients with bridging fibrosis and 43 patients with cirrhosis, 20% developed HCC after 5 years.¹⁹

CURRENT TREATMENT OPTIONS

Lifestyle Modifications

The first-line therapy for the management of steatosis and NASH to date has been lifestyle modification focussing on dietary strategies and increased activity. However, data have been limited, and the biggest impediment is how to measure disease improvement or progression. A randomised study by Harrison et al.²⁰ showed that subjects who lost $\geq 5\%$ of their body weight over 9 months demonstrated increased insulin sensitivity and a reduction in steatosis. In comparison, subjects who lost $\geq 9\%$ of their body weight demonstrated histological improvement.

Bhat et al.²¹ reported that lifestyle modification decreases insulin resistance, resulting in improvements in ALT and liver histology in patients with NAFLD. Diet and exercise have been efficacious in preventing steatosis progression to NASH.²² Villar-Gomez et al.²³ reported that a greater extent of weight loss (induced by lifestyle changes) was associated with a greater level of improvement

in NASH histological features. Weight losses of $\geq 10\%$ in body weight were associated with the highest rates of NAFLD activity score reduction, NASH resolution, and fibrosis regression.²³

There is a general consensus that heavy alcohol intake should be avoided. However, it seems that moderate use of alcohol may have a beneficial effect on NAFLD. Lower rates of steatohepatitis were reported in patients with moderate alcohol drinking compared with non-drinkers.²⁴ The Substance Abuse and Mental Health Services Administration (SAMHSA) defines heavy drinking as consuming ≥ 5 drinks on the same occasion on ≥ 5 of the past 30 days. On the other hand, there is general agreement about the definition of moderate drinking being no more than 3–4 standard drinks per drinking episode, and no more than 9 drinks per week for women and 12–14 per week for men.

Dietary Supplementation

With oxidative stress being a central component to liver injury and damage, antioxidant supplementation has been extensively studied in patients with NASH. Vitamin E (an antioxidant) has been shown to reduce serum ALT levels and improve liver histology.²⁵ Although the randomised controlled PIVENS trial demonstrated that vitamin E can reduce inflammation and ALT levels, no improvement in fibrosis score was observed.^{26,27} Consideration of risk factors must be taken into account before advocating for the use of vitamin E, as studies have suggested that increased vitamin E intake can be associated with a higher risk of developing prostate cancer,²⁸ as well as an increase in all-cause mortality.²⁹ However, more recently, published studies have reported conflicting results.³⁰

Additional antioxidants that have been studied include caffeine and coffee. Both caffeine and coffee have demonstrated the ability to decrease fibrosis, steatohepatitis progression, and are associated with lower instances of NASH amongst users.³¹

While multiple other supplements, including ursodeoxycholic acid, omega-3 fatty acids, probiotics, and high-dose niacin therapy have been studied as potential treatment options for patients with NAFLD, none have produced enough evidence to justify their widespread use. In a recently published meta-analysis, probiotics were shown to lower aminotransferases, total cholesterol, and TNF α , as well as improve insulin resistance in patients with NAFLD.³² In small studies, the efficacy of ursodeoxycholic acid in improving

liver enzymes and other measurable outcomes has been demonstrated. However, significant histologic improvement was not observed in larger studies.³³ Omega-3 fatty acids have shown promise for treating patients with NASH, with several studies utilising omega-3 supplementation demonstrating improvement in ALT levels and hepatic fat content in NASH patients.³⁴ High-dose niacin therapy has revealed an ability to prevent steatohepatitis in murine models.²⁶

Insulin Sensitisers

Insulin resistance plays a key role in the pathogenesis of NAFLD. Several studies of metformin have demonstrated improvement in ALT and insulin sensitivity in patients with NAFLD.^{35,36} However, only a few studies have demonstrated histologic improvement.³⁷ Metformin was found to be less effective in decreasing liver enzymes and hepatic fat content than exercise alone.³⁸ In another study, metformin was reported to produce only a weak improvement compared with diet alone.³⁹ In a controlled study, metformin was found to have little to no histopathologic improvement in patients with insulin resistance and without diabetes.⁴⁰

Other insulin sensitisers such as thiazolidinediones (TZDs), namely pioglitazone and rosiglitazone (pharmacologic activators of peroxisome proliferator-activated receptor gamma [PPAR γ], which is known to be down regulated in models of NASH), have been studied for their efficacy in treating patients with NASH.

A randomised controlled trial (RCT) investigating rosiglitazone (FLIRT 1) reported a 31% improvement in both steatosis and transaminase levels in patients with NASH.⁴¹ In the FLIRT 2 extension trial the results of the previous study were confirmed.⁴² However, when rosiglitazone was supplemented with metformin or losartan, no improvement in histopathology was observed, compared with subjects who took rosiglitazone alone.⁴³

In a study with non-diabetic NASH patients, it was determined that pioglitazone was associated with a reduction in ALT, gamma-glutamyl transferase, and ferritin.⁴⁴ The study also reported reductions in hepatocellular injury, Mallory-Denk bodies, and fibrosis. The reduction in fibrosis associated with pioglitazone therapy in NASH patients was also confirmed in a meta-analysis.⁴⁵

A common side effect reported in all studies was weight gain in patients taking TZDs. The risk of

significant cardiovascular events has led to the withdrawal of rosiglitazone from the market. Rosiglitazone had significantly higher risk of the patient developing congestive heart failure (CHF), myocardial infarction, and death when compared with pioglitazone.⁴⁶ In another study, patients treated with pioglitazone were shown to have a 0.5% higher rate of CHF, compared with the control group.⁴⁷

Statins

Patients with cardiovascular risk factors and disease are commonly prescribed statins as a preventative measure. The GREACE trial, a large prospective study in patients with abnormal liver enzymes and coronary artery disease, concluded that statin treatment is safe, can improve liver tests, and reduce cardiovascular morbidity in patients with mild-to-moderately abnormal liver tests, which are potentially attributable to NAFLD.⁴⁸ Data reported from the St. Francis Heart Study demonstrated a reduction in hepatic steatosis (visualised via computed tomography), in 455 patients receiving combination therapy of atorvastatin and vitamins C and E, compared with placebo.⁴⁹ However, the study did not provide any histological data.

Surgery

According to the National Institute of Health Guidelines, bariatric surgery can be considered for individuals with a body mass index (BMI) >40 kg/m², or with a BMI of 35 kg/m² in patients with obesity-associated comorbidities who have previously tried to lose weight with diet and exercise. The presence of NASH does not seem to increase the complications of bariatric surgery.⁵⁰ Two recent meta-analyses concluded that steatosis, steatohepatitis, and fibrosis appear to improve or completely resolve in the majority of patients following bariatric surgery-induced weight loss.^{51,52}

Currently, the most commonly performed bariatric procedures include Roux-en-Y gastric bypass (RYGB), laparoscopic adjustable gastric band (LAGB), and sleeve gastrectomy, whilst vertical band gastrectomy and biliopancreatic diversion are rarely performed. The majority of follow-up studies examining liver histology after RYGB surgery reported improved hepatic steatosis, inflammation, and fibrosis. However, worsening fibrosis scores were reported in four studies.⁵³ Taitano et al.⁵⁴ followed 160 patients after bypass surgery (median interval 20 months); a decrease in BMI from 52 \pm 10–33 \pm 8 kg/m² was observed, which was

associated with improvements in all major NASH activity scores. Resolution of fibrosis was reported in another cohort of 63 patients who underwent paired liver biopsies between the two procedures and 12 months after surgery.⁵⁵

Thirty-six obese patients who underwent LAGB had paired liver biopsies (mean interval of 25.6 months) before and after the procedure. The weight loss following LAGB surgery was associated with a significant improvement in liver histology.⁵⁶ Significant reductions in aspartate aminotransferase (AST), ALT, triglyceride, and high-density lipoprotein (HDL) levels were reported following laparoscopic sleeve gastrectomy.⁵⁷ However, there are currently no published follow-up studies with histological data.

It is not possible to indicate bariatric surgery exclusively for NASH due to the absence of controlled evidence and long-term data.⁵⁸ Other less invasive options for bariatric therapy, such as intragastric balloons, have demonstrated benefits for liver function, insulin resistance, and histopathologic measures in obese patients with and without NASH.⁵⁹ It is imperative to realise that some patients may have undiagnosed cirrhosis, and as such may need special assessment prior to surgical intervention.

Unfortunately, patients with NAFLD can progress to end-stage liver disease, leaving liver transplantation as the only remaining treatment option. NASH-related cirrhosis is currently the third most common indication for liver transplantation, surpassed only by the hepatitis C virus and alcohol-related cirrhosis, and is anticipated to become the leading indication for liver transplantation within the next one to two decades.⁶⁰ The increasing prevalence of NAFLD in the general population also affects the presence of steatosis in deceased and live donor livers available for transplantation, which can affect the quality and the quantity of the available livers.⁶¹ The outcome of liver transplantation in patients with NAFLD and cirrhosis due to NAFLD are comparable to those transplanted for other causes.⁶² In a recent systematic review and meta-analysis report, 1, 3, and 5-year patient survival were similar in NASH and non-NASH recipients. However, cardiovascular complications and sepsis were more common as causes of death in NASH recipients.⁶³ NAFLD can recur after transplant or develop *de novo*. NAFLD seems to recur in at least one-third of patients who received a liver transplant due to NASH cirrhosis.⁶⁴ While NAFLD recurrence does not

seem to affect overall graft and patient survival for up to 10 years, cardiovascular and infection related morbidity and mortality seem to be increased in these patients.⁶⁵

EMERGING AND POTENTIAL THERAPIES

The fibrates (agonists of PPAR α) are used clinically for the management of dyslipidaemias. A small study (27 patients) in 2010 demonstrated a modest decrease in intrahepatic triglyceride content in obese patients taking fenofibrate compared with placebo.⁶⁶ In another report, a significant reduction in ALT associated with histologic improvement of hepatocellular ballooning degeneration was observed in patients receiving fenofibrate. However, no significant change in the amount of steatosis or fibrosis occurred.⁶⁷

GFT505, a recently developed dual PPAR α / δ agonist, has been shown to reduce hepatic and peripheral insulin sensitivity in obese patients, which is associated with a 20.5% reduction in serum ALT level.⁶⁸ In a short-term (4–8 week) Phase IIa study, GFT505 had a significant lowering effect on the concentration of liver dysfunction markers.⁶⁹ However, in a 1-year study on 274 patients, GFT505 use was not associated with significant improvement in liver fat content or fibrosis.⁷⁰ The results of an international, Phase II RCT of GFT505 in adult patients with NASH demonstrated that a daily dose of 120 mg of GFT505 induced a significant histological improvement and resolution of NASH, compared with the placebo group.⁷¹

Cysteamine bitartrate (RP 103) is an aminothioli antioxidant approved for the treatment of cystinosis. In a 24-week pilot, open-label, Phase IIa clinical trial in 13 children with moderate-to-severe NAFLD, seven patients demonstrated a reduction in liver enzymes in the absence of a significant change in mean BMI. Mean cytokeratin-18 (CK-18) fragment levels decreased by 43% from baseline, while plasma adiponectin levels increased by 31%.⁷²

Obeticholic acid (OCA) is a farnesoid X receptor agonist which has been shown to increase insulin sensitivity and exert anti-inflammatory and antifibrotic effects in preclinical models. A RCT (FLINT trial) of 282 subjects with NASH demonstrated a significantly improved efficacy of OCA relative to placebo in terms of reductions in AST and ALT. A total of 45% of 110 patients in the OCA group demonstrated an improvement in histological features. However, the change did

not reach statistical significance. Compared with placebo, treatment with OCA was also associated with higher levels of total serum cholesterol and low-density lipoprotein cholesterol, and a decrease in HDL cholesterol.⁷³

Simtuzumab is a humanised monoclonal antibody against lysyl oxidase-like 2, an enzyme that is vital to the biogenesis of connective tissue. Simtuzumab is currently in the process of development as an antifibrotic agent. In a study of 20 patients with liver fibrosis of various aetiologies, improvement in transaminases was observed, suggesting a possible anti-inflammatory effect in addition to the antifibrotic effect.⁷⁴ A Phase IIb trial in patients with NASH and compensated cirrhosis is currently underway.

Aramchol is an inhibitor of the activity of stearyl-coenzyme A desaturase 1 in the liver. The inhibition may result in a decrease in storage triglycerides and other esters of fatty acids. In a RCT of 60 patients with biopsy-confirmed NAFLD (including six subjects with NASH), patients on aramchol had their liver fat content decreased by 12.57–22.14%.⁷⁵ There is an ongoing Phase II trial in overweight/obese patients with prediabetes or Type 2 diabetes mellitus and NASH.

Liraglutide is a long-acting glucagon-like peptide-1 receptor agonist approved for the treatment of diabetes and the treatment of obesity in adults with related comorbidity. Liraglutide was reported to be safe and well tolerated. Improvement of liver enzymes was reported in a 26-week trial in patients with diabetes and elevated liver enzymes.⁷⁶

Emricasan is a potent irreversible pan-caspase inhibitor. Caspases play a core role in the processes of apoptosis, inflammation, and activation of cytokines such as interleukin (IL)-1 β and IL-18. All these processes are known to play important roles in the pathogenesis of NAFLD. In a recent 28-day Phase II study in patients with NAFLD and elevated ALT, treatment with emricasan led to statistically significant reductions in ALT and CK-18.⁷⁷

Cenicriviroc is an immunomodulator and dual inhibitor of chemokine receptors CCR2 and CCR5. In patients with HIV, treatment with cenicriviroc was associated with improvements in AST to platelet ratio and fibrosis-4 (FIB-4) scores; correlations were observed between changes in AST to platelet ratio and FIB-4 scores and soluble CD14 levels at Week 48.⁷⁸ Phase II studies in patients with NAFLD are underway.

Remogliflozin has been shown to improve insulin sensitivity in subjects with Type 2 diabetes through SGLT2 inhibition. Treatment with remogliflozin in a 12-week trial in diabetic patients resulted in a 40% reduction in ALT levels in subjects with elevated values at baseline.⁷⁹

Two novel classes of potential pharmacotherapies are the glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors, collectively known as incretin-based therapies, which have several metabolic and anti-inflammatory actions that may be of benefit in NAFLD and Type 2 diabetes mellitus. Treatment with incretin was found to significantly reduce steatosis, inflammation, and fibrosis.⁸⁰

The use of pentoxifylline (anti-TNF α) therapy in NAFLD has also been investigated. Pentoxifylline combined with fenofibrate resulted in beneficial effects on direct and indirect markers of liver fibrosis, liver stiffness, and insulin resistance.⁸¹

NOVEL MOLECULAR TARGETS

In the near future, the identification of key molecular targets that play an important role in the pathogenesis of NAFLD may lead to the development of effective therapeutic options (Table 1).

MCC950, a potent and selective NLRP3 inflammasome inhibitor, has been shown to prevent or reverse inflammation, injury, and fibrosis in two different murine models of NASH. Targeting NLRP3 may be a logical new direction in NASH pharmacotherapy.⁸²

Palmitate (PA), a lipotoxic free fatty acid, is implicated in hepatocyte apoptosis, macrophage-mediated liver inflammation, and activation of the IRE1 α branch of the endoplasmic reticulum stress response. PA-induced extracellular vesicles stimulate macrophage chemotaxis and this may be a mechanism for the recruitment of macrophages to the liver under lipotoxic conditions. It is possible that interference with this macrophage recruitment response may be a therapeutic avenue in NASH.⁸³

NAFLD and obesity are characterised by altered gut microbiota, inflammation, and gut barrier dysfunction. Mucin-2 (Muc2) is the major component of the intestinal mucus layer. It has recently been shown that an impaired gut barrier in Muc2 deficient mice elicits a strong intestinal immune response that is associated with the release of IL-22.

Table 1: Novel therapeutic agents aiming at key molecular targets.

MCC950: Potent and selective NLRP3 inflammasome inhibitor
Palmitate: Lipotoxic free fatty acid
INT-767: Dual agonist of the nuclear receptor farnesoid X receptor and the G-protein coupled receptor
DRX-065: stabilised deuterated R-enantiomer of pioglitazone

Increased systemic IL-22 may mediate beneficial metabolic and anti-inflammatory effects in patients with NAFLD.⁸⁴

INT-767, a dual agonist of the nuclear receptor farnesoid X receptor (FXR) and the G-protein coupled receptor, TGR5. The dual FXR-TGR5 agonist INT-767 has been reported to be able to markedly and significantly arrest and reverse progression of liver disease in mice, even when treatment is started in the presence of obesity, insulin resistance, and NASH.⁸⁵

DRX-065 is a stabilised deuterated R-enantiomer of pioglitazone. DRX-065 has pharmacological

properties desirable for the treatment of NASH (mitochondrial function modulation, non-steroidal anti-inflammatory effects, and glucose lowering effects) without the undesired PPAR γ -related weight gain side effects. Therefore, DRX-065 may represent a potentially significant therapeutic improvement over pioglitazone, a drug already recommended off-label for the treatment of NASH.⁸⁶

CONCLUSION

The problem of obesity has grown tremendously over the last three decades, gradually transforming into an epidemic.⁸⁷ NAFLD has become one of the major diseases plaguing the USA, as well as the rest of the world. The search continues for safe, effective, and affordable therapy for this disorder. The biggest hurdle in the process of developing the ideal therapy is our incomplete understanding of the pathogenesis of the disease. Several therapeutic agents have surfaced over the last few years and though all of these promising agents are still in their infancy, with further research they may become effective therapeutic options. An ounce of prevention is worth a ton of cure. Increased awareness is crucial through public education focussing on lifestyle modification strategies, especially in high-risk groups. Until we reach our goals, nothing is available for NAFLD.

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