

Current treatment paradigms and emerging therapies for NAFLD/NASH

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1. ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) is one the fastest emerging manifestations of the metabolic syndrome worldwide. Non-alcoholic steatohepatitis (NASH), the progressive form of NAFLD, may culminate into cirrhosis and hepatocellular cancer (HCC) and is presently a leading cause of liver transplant. Although a steady progress is seen in understanding of the disease epidemiology, pathogenesis and identifying therapeutic targets, the slowest advancement is seen in the therapeutic field. Currently, there is no FDA approved therapy for this disease and appropriate therapeutic targets are urgently warranted. In this review we discuss the role of lifestyle intervention, pharmacological agents, surgical approaches, and gut microbiome, with regard to therapy for NASH. In particular, we focus the role of insulin sensitizers, thyroid hormone mimetics, antioxidants, cholesterol lowering drugs, incretins and cytokines as therapeutic targets for NASH. We highlight these targets aiming to optimize the future for NASH therapy.

2. INTRODUCTION

Parallel to the global increase in metabolic syndrome, diabetes and obesity, a sharp rise is seen in the prevalence of NAFLD. Non-alcoholic steatohepatitis (NASH) is the clinically serious form of non-alcoholic fatty liver disease (NAFLD), characterized by excessive accumulation of triglycerides (steatosis), inflammation, injury and apoptosis in the liver cells which in extreme cases may lead to cirrhosis and liver cancer (1). NAFLD is currently recognized as the most chronic liver disease, and globally affects around 25% of the adult population (2). It is one the most prominent cause of liver related morbidity and mortality (3).

Owing to its high prevalence and potential risk of progression to cirrhosis and hepatocellular carcinoma (HCC) (4), NASH has become a major health concern worldwide. While it's adverse hepatic consequences include cirrhosis culminating into liver failure and HCC, it has non-liver associated outcomes such as cardiovascular disease (5), (6). The number of NAFLD cases in the United States is

expected to increase from 83.1 million in 2015 to 100.9 million in 2030, a large proportion of which will be NASH (7). This rise will lead to an increase in the number of patients with cirrhosis and end stage liver disease, necessitating liver transplantation (8), (9) and leading to a steep rise in HCC (10).

Despite this high prevalence and growing impact on world health, there are currently no approved treatments for NASH (11). Among all aspects of NASH, the slowest advancement has been seen in the therapeutic field, although a steady progress is seen in understanding disease epidemiology, pathophysiology and identifying therapeutic targets. Even after years of intense research worldwide, presently there is no approved drug for its treatment. Additionally, liver biopsy, a costly and invasive procedure, is still the gold standard for histological evaluation, warranting effective non-invasive imaging and biochemical tests to prevent the need for liver biopsy. This review highlights the treatment strategies for NAFLD/NASH in view of the ongoing rapid progress and unmet challenges, focusing on the critical assessment and understanding of the key elements with regard to therapy for NASH.

3. LIFESTYLE INTERVENTION

Given the fact that presently no drug or surgery is approved for the treatment of NASH, lifestyle modifications (diet, physical activity, and exercise) remain the cornerstone approach for its management. These approaches mainly aim at controlling bodyweight and metabolic disorders (12), (13), (14).

3.1. Physical activity and exercise

Physical activity is recommended for people with NAFLD, as it is a key determinant of metabolic control. People inclined to excessive adiposity, metabolic syndrome and type 2 diabetes (T2D) show higher sedentary behaviour (15). An increase in sedentary time may lead to a predisposition towards NAFLD. Interestingly, the increased breaks to sedentary time are reported to

be beneficial for glucose and fatty acid metabolism, and obesity control (16). Several cross-sectional studies have reported lower levels of physical activity in people with NAFLD (17) (18) (19), and that they are more prone to fatigue (20). Individuals with a healthy lifestyle are less predisposed to develop insulin resistance, diabetes and impaired glucose tolerance (21), (22).

Exercise reduces the possibility of developing NASH (23). It lowers the risk of diabetes, hypertension and metabolic syndrome (24), (25). A meta-analysis reports that exercise reduces hepatic fat content, with little or no weight loss (26). It reduces visceral adipose tissue and plasma free fatty acids. The effect of exercise on histological lesions in NASH is not clearly known, however, preliminary data suggest that exercise has an effect on hepatic markers of apoptosis (27). The effect of physical activity shows a dose-effect relationship (24). Vigorous activity is much more beneficial for NASH and fibrosis, as compared to moderate activity (23). The Compendium of Physical Activities defines specific physical activity (PA) based on the rate of energy expenditure. It defines the intensity of physical activity as the ratio of work metabolic rate to a standard resting metabolic rate (MET). One MET ($4.184 \text{ kJ} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$), is defined as the rate of energy expenditure while a person is at rest or the resting metabolic rate during quiet sitting. Activities having MET values between 3 and 5.9 are classified as moderate activity while those with MET values ≥ 6 are defined as vigorous activities (23).

3.2. Caloric restriction

Excess caloric consumption causes obesity, which is a leading risk factor for NAFLD (2). Caloric restriction (CR) exerts metabolic reprogramming and effective utilization of body fuel, lowering the oxidative damage to cells (28). Carbohydrates (CHO) are the main source of energy in the body. CHO intake is linked to NAFLD (29), and CHO restriction in the diet reduces the glycemic load, and improves insulin resistance and pancreatic β -cell insulin secretion (30). Low CHO diets increase high density lipoprotein (HDL) and reduce serum triglycerides, and glucose (31). In a post hoc analysis of 52 obese and insulin resistant patients, a

hypocaloric low-CHO/high-fat diet (40% and 45% total calories per day, respectively) significantly reduced alanine transaminase (ALT) and serum insulin as compared to a hypercaloric high-CHO/low fat diet (60% and 25%, respectively) (32).

The intake of fructose derived from sugars (table sugar) and corn syrup, commonly used in sweetened beverages, has increased (33). Fructose metabolism enhances free radical production, gut permeability, bacterial growth, lipogenesis, and the level of serum lipopolysaccharides (29), (34). A study reported a significant rise in cholesterol (11.4%), serum triglycerides (32.7%), liver fat (150%), visceral adipose fat (25%), and muscle fat (200%) in 47 overweight patients subjected to a daily intake of 1 litre of sugar-sweetened soft drinks up to 6 months (35). High fructose consumption increases the risk of fibrosis in NASH patients (36). A diet rich in omega-3 polyunsaturated fatty acids (PUFA) reduces intrahepatic triglycerides, increases insulin sensitivity (37), and improves steatohepatitis (38), (39). Therefore, CR is recommended for improvement of NAFLD and the overall calorie intake is recommended to be 1,200-1,500 kcal/day for women and 1,500-1,800 kcal/day for men (40).

3.3. Time restricted feeding

Time-restricted feeding (TRF) constitutes a circadian synchronised dietary approach wherein food access is restricted within a particular time window (8-16 hours) (41). A recent study on NAFLD patients demonstrated that TRF helps to significantly achieve weight loss and reduce triglyceride levels after 12 weeks as compared to the control group (42). Furthermore, TRF regimen mice show reduction in severity of hyperinsulinemia, hepatic steatosis and inflammation, despite the consumption of similar quantities of high-fat diet (HFD) as compared to the ad libitum group (43). Interestingly, TRF also mitigates the effect of maternal HFD feeding on fetal hepatic steatosis (44). However, results from clinical trials to test the efficacy of TRF in human NAFLD/NASH are still awaited.

4. PHARMACOLOGICAL THERAPY

Diet and lifestyle intervention measures cannot be successfully or sustainably implemented in

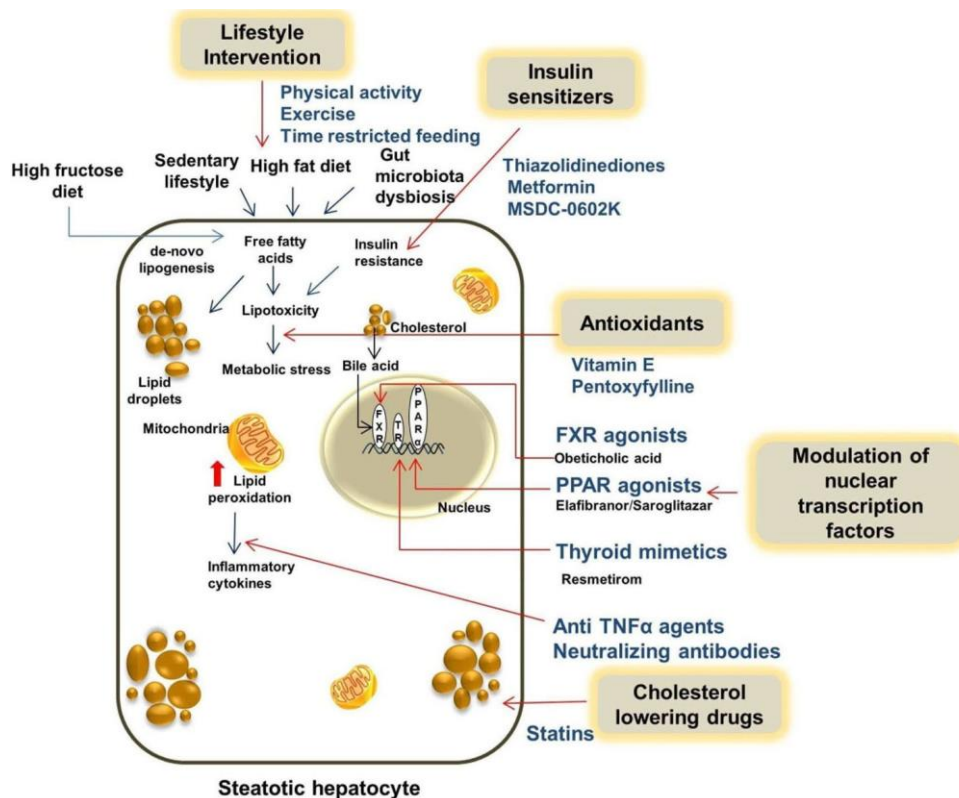


Figure 1. Mechanism of action of lifestyle modification and pharmacological therapy in NAFLD and NASH. NAFLD: non-alcoholic fatty liver disease; NASH: non-alcoholic steatohepatitis; TNF: tumor necrosis factor; FXR: farnesoid X receptor; PPAR: peroxisome proliferator-activated receptor.

most patients. The patients who fail to benefit from lifestyle intervention or those with already advanced disease (significant fibrosis), need pharmacological treatments which are specifically aimed at improving hepatic inflammation, fibrosis and steatohepatitis (45), as illustrated in Figure 1. Steatohepatitis increases liver-related mortality and reduces survival by progression into cirrhosis, liver failure and HCC, therefore, pharmacological therapy is restricted to patients with NASH (46), (45), (47). Several randomized controlled trials (RCTs) have tested pharmacological agents, which show histological improvements in NASH. An effective therapy for NASH is still in infancy; well conducted RCTs aimed at relevant therapeutic targets are needed.

4.1. Insulin sensitizers

Insulin resistance is a major driving force for excessive fat accumulation in the liver and plays

a key role in the initiation and progression of steatohepatitis and fibrosis. Several pharmacological agents listed below are focused to improve insulin resistance.

4.1.1. Thiazolidinediones

Thiazolidinediones (TZD), also known as glitazones, are the strongest evidence-based drugs tested for the treatment of NASH (47). Current guidelines recommend the use of glitazones in NASH (45), (46). Glitazones promote the differentiation of insulin-resistant large pre-adipocytes into small, proliferative, insulin-sensitive adipocytes (49) (50) (51). Upon the induction of lipogenic genes (52) and lipoprotein lipase (LPL), they elevate fatty acid synthesis and uptake in adipose tissue (53), thereby diverting the free fatty acid (FFA) burden towards adipocytes, instead of the liver. Despite the expansion in fat mass, insulin sensitivity is subsequently improved. Alternatively, glitazones

upregulate the production of the insulin-sensitizing and anti-steatogenic adipokine, adiponectin (54), which enhances fatty acid beta-oxidation in liver and muscles (55). Thus, they act by redistributing fat from ectopic tissues to the adipose tissue, and by increasing levels of adiponectin. They also contribute to the reduction of insulin resistance by increasing the expression of AMP-activated protein kinase (56), (57) and upregulating glucose transporter type -4 (GLUT-4) in muscle and adipose tissue.

Some studies based on rodents also report anti-inflammatory actions of glitazones, showing decreased hepatic fibrogenesis in response to recurrent liver injury (58), (59). Interestingly, these studies show a reduction in the nuclear expression of peroxisome proliferator-activated receptor (PPAR)- γ , upon activation and trans-differentiation of stellate cells into fibrogenic myofibroblasts (58). PPAR- γ expression was restored upon treatment with PPAR- γ agonists and hepatic scar-forming response was elevated (60). This suggests a hepatoprotective effect of glitazones in addition to their insulin-sensitizing property. Rosiglitazone and pioglitazone are the two available glitazones, which exhibit similar cellular action, except lipoprotein metabolism which favours pioglitazone (61). The widespread use of glitazones is restricted by their safety concerns and adverse effects including congestive heart failure (62), bone fractures in women (63), and increased risk of bladder cancer for pioglitazone (64), owing to which, it was withdrawn from the market in France and Germany. As of now, the European Medicines Agency and the FDA continue the licence pioglitazone, except in patients with, or at the risk of developing bladder cancer. Pioglitazone is recommended for treatment of steatohepatitis in biopsy-proven NASH patients. However, the long term benefits and safety issues remain unknown.

4.1.2. Metformin

Another insulin-sensitizing agent is metformin, an oral biguanide, which reduces hepatic glucose production and increases peripheral glucose utilization (65). Metformin is approved for use in patients with type T2D (66). It causes a reduction in endogenous glucose production, activates AMP-activated protein kinase (67) and inhibits the mitochondrial glycerophosphate dehydrogenase

shuttle with change in redox state (46). Metformin is considered as a safe drug, however, it is not recommended for the treatment of NASH (69) as there is no proof of its efficacy on hepatic histology.

4.1.3. MSDC-0602K

Pioglitazone, the first-generation insulin sensitizer improved NASH but proved to be of little use owing to its side effects (68). Another second-generation insulin sensitizer, MSDC-0602K, an inhibitor of mitochondrial pyruvate carrier (MPC) with PPAR- γ binding, is reported to enhance lipid oxidation and downregulate de novo lipid synthesis and gluconeogenesis in liver, *in vivo* and *in vitro*. MSDC-0602K does not exhibit the side effects associated with the first generation insulin sensitizers (68). MSDC-0602K leads to hepatic histological improvement and reduction in ballooning or lobular inflammation with no increase in fibrosis at 12 months, as reported in the phase 2b, 52-week double-blind study (68).

4.2. Antioxidants

Increased oxidative stress and defected antioxidant defence mechanisms have been studied extensively across the progressive stages of NAFLD, and are believed to contribute to liver injury and development of NASH. Some of the agents with antioxidant activity being used as a therapy for NASH are listed below.

4.2.1. Vitamin E

Vitamin E is a fat soluble compound present in a variety of compounds which belong to two main families, tocopherol and tocotrienol. Vitamin E is present in the phospholipid bilayer of the cell membranes, where it helps to protect against oxidative damage caused by free radicals (55). It protects against mitochondrial toxicity and blocks intrinsic apoptotic pathways, thereby protecting against liver injury (69), (70). It is also reported to have non-antioxidant properties and may alter cell signaling, gene expression (71) or down-regulate nuclear factor kappa B (NF- κ B) dependent inflammatory pathways (72).

The largest RCT until now, the PIVENS trial (73) where vitamin E (800 IU/day) was tested along

with pioglitazone and placebo in non-diabetic and non-cirrhotic patients with NASH for 2 years, showed significant histological improvement in patients. Vitamin E improved steatosis, inflammation and ballooning, and resolution of NASH was induced in 36% of the patients receiving vitamin E. The safety concerns related to vitamin E include overall mortality (74), haemorrhagic stroke (75), and prostate cancer in males older than 50 years (76). However, the long term safety of vitamin E remains a concern.

4.2.2. Pentoxifylline

Pentoxifylline is a non-selective phosphodiesterase inhibitor used for the treatment of peripheral vascular disease. Animal studies report that pentoxifylline exhibits antioxidant effect (77) and inhibits the production of pro-inflammatory cytokines (78). These effects were confirmed *in vivo*, where pentoxifylline significantly decreased steatohepatitis in methionine-choline-deficient dietary NASH model (79). In a 1 year long RCT in 55 patients with NASH receiving pentoxifylline or placebo, pentoxifylline improved liver enzyme levels and histological features of NASH, and displayed a marginal effect on fibrosis (80). Good quality human data regarding the use of pentoxifylline in NASH are lacking.

4.3. Lipotoxicity based targets

The progression from steatosis to NASH may be determined by the accumulation of lipotoxic intermediates of triglyceride synthesis and/or lipolysis (81), (82). De novo lipogenesis (DNL) refers to the biochemical process of synthesis of fatty acids from acetyl-CoA subunits commonly produced by carbohydrate catabolism, which occurs primarily in the liver. Fructose is a profound lipogenic substrate which drives DNL, in addition to glucose. Increased hepatic DNL contributes significantly to NASH. The rate-limiting step in DNL is catalysed by acetyl-CoA carboxylase (ACC), which occurs in two major isoforms in mammals, ACC1 and ACC2. The pharmacological inhibition of ACC is another attractive approach to control fatty acid synthesis in lipogenic tissues. GS-0976 is an inhibitor of acetyl-CoA carboxylase. In an open-label study, NASH patients administered with GS-0976 for 12 weeks showed reduction in hepatic DNL, hepatic steatosis

and improvement in markers of liver injury (ClinicalTrials.gov no: NCT02856555) (83). NDI-010976, an allosteric inhibitor of ACC1 and ACC2, reduces hepatic de DNL and is reported to improve steatosis, inflammation, and fibrosis in animal models with fatty liver disease (84).

4.3.1. Cholesterol lowering drugs

Cholesterol is a key toxic lipid mediator of liver injury, which may be targeted by specific pharmacological interventions. The synthesis of mono-unsaturated fatty acids such as oleic acid is catalysed by the enzyme stearoyl-CoA desaturase 1 (SCD-1) (85). The deficiency or inhibition of SCD-1 leads to a reduction in liver steatosis and improvement in insulin sensitivity (86), (87), (88). Enhanced SCD-1 activity has been reported in obese patients with NASH (89). A double blind placebo-controlled clinical trial in 60 NAFLD patients reported a reduction in hepatic fat content with the administration of Aramchol, the SCD-1 inhibitor (90).

Statins, also known as HMG-CoA reductase inhibitors, are a class of cholesterol lowering drugs. Statins are reported to show marginal improvement of steatosis (91). They are the first choice agents used to reduce plasma cholesterol and reduce cardiovascular disease (CVD) risk (92). CVD is a leading cause of death in patients suffering from NAFLD and NASH (93). Statins do not show any beneficial effect on liver histology in patients with NASH (91). Therefore, statins are not currently recommended for NASH treatment (46). Concurrent metabolic comorbidities recommend the use of statins for cardiovascular end points in NAFLD patients. A number of studies have focused on the safety of statins in hepatotoxicity (94) as these drugs are reported to lower aminotransferase levels in patients with the metabolic syndrome (95).

4.4. Modulation of nuclear transcription factors

Nuclear transcription factors are also believed to have therapeutic potential for the treatment of NASH. These are molecules, which upon binding to their specific ligand regulate transcription of specific genes.

4.4.1. Farnesoid X receptor (FXR) agonists

The farnesoid X receptor (FXR) is a member of the nuclear receptor superfamily, and has emerged as a key regulator of metabolic pathways including glucose homeostasis and the inflammatory and fibrogenic processes. The hepatic expression level of FXR is inversely correlated with the severity of disease in patients with NASH (96). The FXR is expressed in the liver, kidneys, intestines, and adrenal glands. It is involved in the synthesis and enterohepatic circulation of bile acids and regulates lipoprotein metabolism [97]. FXR activation affects bile acid production, hepatic lipogenesis, cholesterol synthesis and glucose homeostasis, and protects hepatocytes against bile acid-induced cytotoxicity [98, 99]. This finding is corroborated by FXR-deficient mice fed on a high-fat diet, which exhibit severe hepatic steatosis, necrotic inflammation and fibrosis (100). Furthermore, FXR agonists have been shown to prevent the development of NASH in rodent models of diet-induced NASH, where they also promoted the resolution of steatohepatitis and fibrosis (101). Therefore, a number of synthetic FXR agonists are being tested for the treatment of NASH and other hepatic disorders (102), (103).

4.4.1.1. Obeticholic acid

Obeticholic acid (OCA) is a modified bile acid which is derived from the primary human bile acid, chenodeoxycholic acid. It stimulates the FXR in humans (104). The FXR plays a key role in the synthesis and entero-hepatic circulation of bile acids (105) and is expressed in the liver, adrenal glands, kidneys and intestines. OCA is a synthetic 6 α -ethyl derivative of chenodeoxycholic acid, the primary human bile acid. It is a potent agonist of the FXR, a nuclear hormone receptor which regulates glucose and lipid metabolism. This chemical modification stimulates the activity of FXR approximately 100-fold more than the natural FXR agonist in humans (106), chenodeoxycholic acid. It shows high selectivity and minimal activity with other bile acid receptors (107).

The FLINT trial, a phase 2b study (108) and double-blind, placebo-controlled, randomized clinical trial in the USA, assessed NASH patients without cirrhosis and a NAFLD activity score (NAS) ≥ 4 , for 72 weeks. Improvement in centrally scored liver histology by 2 points in NASH was observed, without

any worsening of fibrosis. In FLINT trial, the administration of OCA was associated with significantly great increase in the concentration of total cholesterol and low-density lipoprotein cholesterol (LDLc) and decrease in the concentration of high-density lipoprotein cholesterol (HDLc) as compared to placebo. However, the increase in LDLc concentrations observed upon treatment with OCA were reversed with concomitant statin therapy (e.g. atorvastatin).

Another ongoing phase 3 REGENERATE trial (109) aims to compare the effects of OCA and placebo in the histological improvements and clinical outcomes in NASH and liver fibrosis stage 2 and 3. The patients are randomized and treated with OCA 10 mg, OCA 25 mg and placebo. The primary analysis included 931 patients suffering from F2-F3 fibrosis (311 placed in the placebo group, 312 in OCA 10 mg treated group and 308 in OCA 25 mg treated group). An improvement in fibrosis was seen in 37 (12%) patients of the placebo group, 55 (18%) patients of the OCA 10 mg group ($P = 0.045$), and 71 (23%) patients of the OCA 25 mg group ($P = 0.0002$). The resolution of NASH was not attained in any of the patients.

4.4.1.2. Tropifexor (LJN-452)

Tropifexor (LJN-452) is a very potent non-bile acid agonist of FXR, which is reported to induce target genes at very low concentrations. Tropifexor has been shown to be very effective in preclinical NASH models (110). An ongoing phase 2 RCT, FLIGHTFXR, is evaluating the efficacy and safety of tropifexor and cenicriviroc in patients with fibrosis and biopsy-proven NASH (111).

4.4.2. Peroxisome proliferator-activated receptors (PPARs)

PPARs are a group of nuclear receptors, which are expressed in the liver, adipose tissue, heart, skeletal muscle and kidney, and transcriptionally regulate multiple metabolic processes including β -oxidation, lipid transport and gluconeogenesis (112). They are classified into three isotypes, designated as PPAR α , PPAR β (also known as PPAR δ) and PPAR γ , which differ in tissue distribution; however, they target the same segment of DNA. The PPARs are known to heterodimerize

with the retinoid X receptor (RXR) to regulate target gene transcription.

In the liver, PPAR α upregulates several enzymes involved in mitochondrial and peroxisomal fatty acid oxidation, microsomal ω -oxidation, and ketogenesis (113), (114), and therefore shifts the hepatic metabolism towards lipid oxidation. The activation of PPAR α upregulates the expression of lipoprotein lipase (LPL) while downregulating the hepatic secretion of APOCIII, a LPL inhibitor (115), thereby elevating plasma triglyceride clearance. *In vitro* and *in vivo* studies report that PPAR α suppresses the secretion of interleukin 1 (IL-1), IL-6 and tumour necrosis factor (TNF), and intercellular adhesion molecule 1 (ICAM1) and vascular cell adhesion molecule 1 (VCAM1) independent of direct DNA binding (116), (117). These effects were peroxisome proliferator response elements (PPRE)-independent, yet they were hepatoprotective against methionine–choline-deficient diet-induced inflammation and fibrosis, without altering fatty acid oxidation and lipid accumulation (118).

4.4.2.1. Elafibranor

Elafibranor is a PPAR-alpha/delta (α/δ) agonist, known to regulate lipid and insulin metabolism in NAFLD and NASH (119). A phase 2b, double blind RCT, the GOLDEN study (Europe and USA), compared 80 mg and 120 mg elafibranor daily to placebo in 276 patients with biopsy-proven, non-cirrhotic NASH with NAS ≥ 3 and ≥ 1 point, for 52 weeks. NASH was resolved, without the fibrosis worsening in the higher proportion of patients of the 120 mg elafibranor group as compared to the placebo group (19% vs 12%; odds ratio = 2.31; P = .045). However, reversal of NASH was not achieved (119).

An ongoing phase 3 study, RESOLVE-IT (<https://clinicaltrials.gov/ct2/show/NCT0270443>) includes 2,000 patients with NASH having NAS ≥ 4 , with ≥ 1 of each component of score, and F1-F3 fibrosis. The trial aims at the histological improvement in NASH, evident by the resolution of NASH without any worsening of fibrosis at 72 weeks, and to evaluate cirrhosis, liver related clinical outcomes, and mortality at 4 years. The results of this trial are due in December 2021.

4.4.2.2. Saroglitazar

Another dual-PPAR agonist, saroglitazar, has been shown to improve lipid and glucose parameters by predominant PPAR- α and moderate PPAR- γ agonist activity. A phase-2 RCT determined the efficacy of saroglitazar magnesium as compared to placebo in patients with NASH (120). A significant reduction was observed in mean ALT levels from baseline to week 16, with 1 mg (–27.3%), 2 mg (–33.1%) and 4 mg (–44.3%) saroglitazar as compared to placebo (4.1%) (P < 0.001 for all groups). A high proportion of patients showed $\geq 50\%$ reduction in the mean ALT levels from the baseline to week 16 with 4 mg saroglitazar as compared to the placebo (51.8% versus 3.5%; P < 0.0001). At week 16, a significant proportion of patients showed $>30\%$ reduction in the liver fat content with 4 mg saroglitazar as compared to placebo (40.7% vs 8%, P = 0.006).

4.4.3. Thyroid hormone receptor (THR)

Thyroid hormone receptor β (THR- β) is known to be highly expressed in the hepatic cells. It plays a key role in regulation of the metabolic pathways which are known to be damaged in NASH (121). Animal studies have reported a crucial role of THR- β in reducing triglycerides and cholesterol, improving insulin sensitivity, promoting liver regeneration, and reducing apoptosis. Evidences indicate that NASH may be, in part, caused by hepatic hypothyroidism, which is higher in patients with NASH (121), (122).

4.4.3.1. Resmetirom (MGL-3196)

Encouraged from the earlier preclinical studies Thyroid hormone mimetics are being actively pursued as possible anti-NASH therapy (123). Resmetirom (MGL-3196) is an orally active agonist of THR. It is liver-directed and about 28 times more selective for THR- β versus THR- α than triiodothyronine (124). In NASH, this selectivity for THR- β is believed to provide metabolic benefits of thyroid hormone which are mediated by the liver, while avoiding the unwanted effects of excess thyroid hormone in the heart and bone which are mainly mediated through THR- α (121). In preclinical animal models of NASH, resmetirom and some other thyroid analogues were shown to reduce hepatic triglycerides, hepatic steatosis, lipid peroxidation,

inflammatory and fibrosis markers, along with ALT levels (124), (121). A multicentre, randomised, double-blind, placebo-controlled, phase clinical 2 trial based on Resmetirom treatment (ClinicalTrials.gov Identifier: NCT03900429) resulted in the significant reduction of hepatic fat in patients with NASH, after 12 weeks and 36 weeks of treatment. The adverse effects of treatment in study groups were mild or moderate, except high occurrence of transient mild diarrhoea and nausea observed in the group treated with Resmetirom (125).

VK2809 is another tissue and receptor subtype selective agonist of THR- β which is specific to the liver tissue and hold promising therapeutic potential. It belongs to the pro-drug family which are cleaved *in vivo* and release potent thyromimetics. It is believed to improve cholesterol and lipoprotein levels through multiple mechanisms including induction of gene expression of genes associated with lipid metabolism. A phase 2, randomized, double-blind, placebo-controlled, multicenter study has been designed to assess the efficacy, safety, and tolerability of VK2809 in lowering LDL-C and liver fat content when administered in patients with primary hypercholesterolemia and NAFLD for 12 weeks, followed by a 4-week off-drug phase (ClinicalTrials.gov Identifier: NCT02927184). Patients receiving 5mg daily doses of VK2809 demonstrated a statistically significant median reduction of 53.8% in liver fat. Of the patients receiving VK2809, 88% showed hepatic fat reduction $\geq 30\%$ at 12 weeks. At all doses studied, VK2809 was safe and well tolerated and no adverse effects were reported among patients (126).

4.5 Gastrointestinal hormones (Incretins)

Evidences indicate that peptide hormones from the Langerhans cells of distal small intestine serve as incretins, which trigger the release of insulin by the pancreatic beta cells. These gut derived incretin hormones are potential targets for the treatment of NASH.

4.5.1. Glucagon-like peptide (GLP)-1 agonist

Glucagon-like peptide (GLP)-1 is an intestinal hormone released from the foregut in

response to food consumption. GLP-1 exhibits a glucose lowering action by its glucose-dependent ability to stimulate insulin secretion and inhibit glucagon secretion from the pancreas. It also delays gastric emptying and suppresses appetite. These metabolic effects of GLP-1 contribute to its anti-NASH activity (127). Its endogenous form is, however, rapidly degraded *in vivo* by the enzyme dipeptidyl peptidase-4 (DPP-4). GLP-1 secretion is reported to be impaired in patients with NAFLD and NASH, suggesting potential role of GLP-1 agonists in NAFLD treatment (128). The GLP-1 agonists mimic gastrointestinal endogenous GLP-1 hormone and have increased resistance to DPP-4 (129). They have a beneficial effect on insulin resistance and weight control (130). The presence of GLP-1 receptor in hepatocytes has been reported by several studies; therefore GLP-1 agonists may exert their direct effect on liver. Studies suggest that GLP-1 agonists reduce fat accumulation by activating macroautophagy and chaperone mediated autophagy (131).

A pilot study reports Liraglutide, a GLP-1 agonist, to improve NASH histology upon daily injection (132). Semaglutide is another GLP-1 agonist under study (NCT02970942), having the advantage that it requires weekly dosing only. Therapies for NASH, aimed at using a combination of GLP-1 with glucagon or GLP-2 are in early development stages.

4.6. Cytokines

Cytokines are the cell signaling molecules produced by a variety of cells in the body, including liver cells (133). They are crucial mediators of inflammation related disorders. They include several subfamilies such as including interferons, interleukins (IL), transforming growth factors (TGF), TNF, colony-stimulating factors, and chemokines. Lipid accumulation and inflammation are crucial in NAFLD; therefore, cytokines may play a key role in the pathogenesis of NAFLD by stimulating hepatic inflammation, steatosis, cell apoptosis and necrosis, and by inducing fibrosis. The pro-inflammatory cytokines, IL family and TNF α are reported to control the key features of liver disease.

4.6.1. Tumor necrosis factor-alpha (TNF- α)

TNF- α is a pro-inflammatory cytokine secreted by monocytes/macrophages, neutrophils, and T-cells, and other tissues, such as the adipose tissue, neuronal tissue and endothelium. It is associated with obesity and related insulin resistance (134). Dietary and genetic (*ob/ob*) mice models of obesity lacking TNF α showed improved insulin sensitivity (135). Clinical investigations show that TNF α expression is increased in obesity and decreases on weight loss (136), (137) indicating its role in obesity and metabolic dysregulation (138). A Spanish study based on 52 obese patients showed that liver disease severity correlated with TNF- α gene expression in adipose and liver tissue (139). Overexpression of TNF- α mRNA was observed in the adipose tissue and liver of NASH patients. An Italian study reported increased prevalence of TNF- α polymorphism in NAFLD patients as compared to the controls (140). Hence, TNF α is proposed as a potential therapeutic target to prevent the consequences of metabolic syndrome.

The anti-TNF α drug, thalidomide helped to improve the hepatic alterations caused by a high fat diet in mice (141). Anti-TNF α antibodies decreased inflammation, necrosis, and fibrosis in an experimental rat model of NASH (142). The neutralization of TNF α by Infliximab showed substantial reduction in steatosis, in *ob/ob* mice (143). Although animal models of NAFLD show encouraging therapeutic potential of TNF- α inhibition, its role in humans is controversial. Hotamisligil *et al* (144) reported a relationship between the expression of TNF- α and insulin resistance in NASH. A positive correlation has been reported between circulating TNF- α levels and the degree of liver fibrosis, in patients with NASH (144). Increased expression of TNF- α has been observed in the liver and adipose tissue of NASH patients showing significant fibrosis as compared to those with slight or non-existent fibrosis (145). Hui *et al* (146) showed increased TNF α levels in steatohepatitis subjects as compared to the controls. However, some studies showed no correlation between insulin resistance and TNF α levels (147), (148).

4.6.2. Transforming growth factor-beta (TGF- β)

Transforming growth factor-beta (TGF- β) is an immunosuppressive and pro-fibrotic cytokine (149). TGF- β 1 mediates the activation of stellate cells and plays a key role in hepatic fibrosis (150) (151) (152). Its expression is upregulated in experimental hepatic fibrosis models (153), (154), (155). Furthermore, the TGF- β 1 mRNA expression is increased in patients with liver fibrosis (156), (157), (158). Stärkel *et al* (159) reported that upregulation of TGF- β 1 is an early step in progressive fibrotic steatohepatitis. Hasegawa and co-workers showed that the levels of TGF- β 1 are increased in NASH patients. Serum TGF- β 1 levels may help to distinguish NASH patients from the spectrum of NAFLD (160). Liver fibrosis is believed to be a strong predictor of mortality in patients with NAFLD, therefore, there is growing interest in developing therapeutics to target elements in fibrogenesis. TGF- β is a key pro-fibrogenic cytokine, which may be a promising target to treat fibrosis. High levels of TGF- β , caused by chronic liver damage, result in activation of stellate cells to myofibroblasts and massive hepatocyte cell death contributing to the promotion of liver fibrosis. TGF- β signaling induces Smad-dependent and TAK1-dependent signaling which regulates cell survival, proliferation, fibrosis, and tumorigenesis [161]. Galunisertib (LY2157299) is a promising anti-fibrotic TGF- β inhibitor which inhibits SMAD2 phosphorylation and blocks collagens deposition [162]. Although several approaches to inhibit TGF- β signalling in animal models have yielded promising results, the complex role of TGF- β in the liver in cell proliferation, carcinogenesis and immune modulation is a major problem [163, 164] to developing anti- TGF- β based therapy for NASH.

Cenicriviroc (CVC) is a dual antagonist of chemokine receptor type 2 (CCR2) and type 5 (CCR5), located on hepatic stellate cells and Kupffer cells is being targeted for anti-fibrosis therapy [165]. It blocks overactive inflammatory signalling and disrupts signalling that activates stellate cells thereby targeting both inflammation and fibrogenesis. CVC is orally administered in daily tablets (150 mg) and has a plasma life of 30-40 hours. CENTAUR, a phase 2b, 24-month study included 189 patients with biopsy-

proven NASH, with an NAS ≥ 4 and stage 1-3 fibrosis. The patients were randomized 2:1:1 in three arms as follows: A) continuous administration of 150-mg CVC for 24 months, B) administration of placebo for 12 months followed by an additional 12 months of CVC, and C) administration of placebo for 24 months. The expected primary outcome of ≥ 2 point decrease in NAFLD activity score without any worsening in fibrosis at 1 year was not achieved. However, the improvement in liver fibrosis without worsening of NASH was achieved in 20% from the treatment group, along with lower levels of interleukin-6, C-reactive protein and fibrogen [166]. Currently, another randomized, double-blind, placebo-controlled, multi-centre phase 3 study, AURORA (<https://clinicaltrials.gov/ct2/show/NCT03028740>) is evaluating the safety and efficacy of CVC in treatment of fibrosis, in NASH.

4.6.3. Interleukin-11 (IL-11)

Hepatic stellate cells (HSCs) give rise to upto 95% of liver myofibroblasts (167), and are crucial to fibrosis, inflammation and parenchymal dysfunction in NASH (168) (169). They are a major source of proinflammatory myofibroblasts, and therefore, their inhibition or reversal of transformation in the liver, may be a potential therapy for treatment of NASH. Interleukin-11 (IL11) was recently identified as a key factor for pulmonary and cardiovascular fibroblast-to-myofibroblast transformation (170, 171). ERK-dependent IL11 signaling is critical for HSC transformation (154), (155), (172).

Recently, a study by Widjaja *et al* based on hepatocytes, HSCs, and mouse models, explored the role of IL-11 signaling in NASH pathogenesis (172). They reported that HSCs express high levels of interleukin 11 subunit alpha (IL11RA) and showed a proinflammatory role of IL11 in the liver. The ALT levels reversed from approximately 700 U/L to normal within 3 weeks, upon pharmacologic targeting of IL11 signaling (172). They showed that neutralizing antibodies which block IL11 signaling, caused reduction in fibrosis, steatosis, hepatocyte apoptosis and inflammation, in mice with diet-induced liver steatosis and fibrosis. They also reported that hepatocyte damage is prevented, or reversed, by genetic or antibody-mediated inhibition of IL11.

Therefore, targeting IL11 to reverse liver fibrosis may be a beneficial therapy for NASH.

5. BARIATRIC SURGERY

Bariatric surgery or a weight loss surgery may be opted to achieve weight loss in NAFLD/NASH patients. Weight loss through diet and exercise may be difficult to achieve or sustain. Bariatric surgery helps to achieve long-term weight loss. It also helps to improve the metabolic functioning of lipid metabolism and inflammatory pathways associated with pathophysiology of NAFLD (173), (174). Bariatric surgery is currently recommended in patients with BMI ≥ 40 kg/m² having no comorbidities, or with a BMI 35 to 39.9 kg/m² with any serious comorbidity such as T2D, hypertension, NAFLD and NASH (175), (176). A review based on 29 studies showed significant improvement in ALT, aspartate aminotransferase (AST), Gamma-glutamyl transferase (GGT) and histology in patients undergoing bariatric surgery (177). A meta-analysis reported improvement in steatosis, steatohepatitis, and fibrosis following weight loss after bariatric surgery (178). Bariatric surgery is an invasive procedure and is therefore associated with risks such as adjustable gastric banding (AGB), and dietary complications including nutritional deficiencies, postprandial hyperinsulinemic hypoglycaemia, etc. (179), (180), (181), (182), (183). A review by le Roux and Heneghan has outlined the long term complications associated with bariatric surgery (179).

6. GUT MICROBIOME

The gut microbiome produces substances which help to regulate immunity, nutrition, homeostasis, and several metabolic pathways (184), (185), (186). The gut microbiota interact with liver through the 'liver-gut axis', involving specific metabolites such as bile acids (BAs), short-chain fatty acids (SCFAs), and lipopolysaccharides (LPS) (187). Several bacteria are known to be associated with lipid and carbohydrate metabolism. Evidences indicate the effect of gut microbiota on liver function, which contribute to energy metabolism, obesity, T2DM and NAFLD (188), (189). Therefore, gut microbiota may regulate disorders linked to energy metabolism (184), (190), (191) Gut microbiota

dysbiosis may be caused by the changes in the environment of the host such as diet, alcohol intake, genetic factors, and antibiotics (192). Gut microbiota dysbiosis plays a pivotal role in the pathogenesis of metabolic disorders including NAFLD (193)

Studies based on animal models suggest that probiotics, prebiotics and synbiotics have a modulatory effect on gut microbiota. The oral administration of probiotics is shown to improve abnormal lipid metabolism and dysbiosis of gut microbiota (194). Supplementation of prebiotics and synbiotics improved hypercholesterolemia-associated hepatic changes in rats by regulating genes involved in β -oxidation and lipogenesis, including PPAR- α , carnitine palmitoyltransferase 1 (CPT-1), sterol regulatory element-binding protein 1c (SREBP-1c), fatty acid synthase (FAS) and malic enzyme (ME) (195). Improvement in hepatic inflammation and insulin resistance was observed in rats treated with synbiotics. Also, a reduction in the amount of Gram-negative *Enterobacteriales* and *Escherichia coli* was seen in the colonic mucosa.

Fecal microbiota transplantation (FMT) is a method for transplantation of fecal bacteria from a healthy donor to re-populate gut microbiota in patients for the treatment of several diseases (196), (197), (198). FMT is being widely explored for the treatment of NAFLD/NASH. Intrahepatic lipid accumulation, insulin resistance and pro-inflammatory cytokines were improved in mouse models, by FMT (199), (200). In a RCT based on patients with metabolic syndrome, increase in insulin sensitivity was observed after 6 weeks of receiving gut microbiota from healthy Caucasian males (201). FMT may also be developed as a potential therapeutic method for NASH.

7. SUMMARY

While there is no FDA-approved medication for NAFLD/NASH, dietary and lifestyle intervention is the mainstay of treatment. Several medications are in pipeline of therapy of NASH, holding promise for a successful therapy in future. As of now, the first line drugs such as pioglitazone and vitamin E remain the strategy for disease management in patients.

8. ACKNOWLEDGMENTS

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Abbreviations: NASH: non-alcoholic steatohepatitis; NAFLD: non-alcoholic fatty liver disease; HCC: hepatocellular carcinoma; CR: caloric restriction; CHO: carbohydrates; HDL: high density lipoprotein; ALT: alanine transaminase; PUFA: polyunsaturated fatty acids; TRF: time-restricted feeding, HFD: high-fat diet; RCT: randomized controlled trial; TZD: thiazolidinediones; LPL: lipoprotein lipase; FFA: free fatty acid; PPAR: peroxisome proliferator-activated receptor; GLUT-4: glucose transporter type-4; T2D: type 2 diabetes; MPC: mitochondrial pyruvate carrier; NF- κ B: nuclear factor kappa B; SCD: stearoyl-CoA desaturase, CVD: cardiovascular disease; FXR: farnesoid X receptor; OCA: obeticholic acid; TNF: tumour necrosis factor; VCAM1: vascular cell adhesion molecule 1; IL-1: interleukin 1; ICAM1: inter-cellular adhesion molecule 1; THR: thyroid hormone receptor; GLP-1: glucagon-like peptide; DPP-4: dipeptidyl peptidase-4; TGF: transforming growth factors; HSCs: Hepatic stellate cells; IL-11: Interleukin-11; AGB: adjustable gastric banding; Bas: bile acids; SCFAs: short-chain fatty acids; CPT-1: carnitine palmitoyltransferase 1; SREBP-1c: sterol regulatory element-binding protein 1c; FAS: fatty acid synthase; ME: malic enzyme; FMT: Fecal microbiota transplantation

Key Words: NAFLD, NASH, Therapy, Insulin Sensitizers, Antioxidants, Statins, Incretins, Cytokines, Bariatric Surgery, Gut Microbiome, Review

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