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TOPIC HIGHLIGHT

WJG 20th Anniversary Special Issues (12): Nonalcoholic fatty liver disease

Focus on emerging drugs for the treatment of patients with non-alcoholic fatty liver disease

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Abstract

Non-alcoholic fatty liver disease (NAFLD) has become the most common liver disorder in Western countries and is increasingly being recognized in developing nations. Fatty liver disease encompasses a spectrum of hepatic pathology, ranging from simple steatosis to non-alcoholic steatohepatitis, cirrhosis, hepatocellular carcinoma and end-stage liver disease. Moreover, NAFLD is often associated with other metabolic conditions, such as diabetes mellitus type 2, dyslipidemia and visceral obesity. The most recent guidelines suggest the management and treatment of patients with NAFLD considering both the liver disease and the associated metabolic co-morbidities. Diet and physical exercise are considered the first line of treatment for patients with NAFLD, but their results on therapeutic efficacy are often contrasting. Behavior therapy is necessary most of the time to achieve a sufficient result. Pharmacological therapy includes a wide variety of classes of molecules with different therapeutic targets and, often, little evidence supporting the real efficacy. Despite the abundance of clinical trials, NAFLD therapy remains a challenge for the scientific community, and there are no licensed therapies for NAFLD. Urgently, new pharmacological approaches are needed. Here, we will focus on the challenges facing actual therapeutic strategies and the most recent investigated molecules.

Key words: Non-alcoholic steatohepatitis; Non-alcoholic fatty liver disease; Fatty liver; Steatosis; Emerging drugs

Core tip: At the moment, there is no standardized treatment for non-alcoholic fatty liver disease (NAFLD) or non-alcoholic steatohepatitis (NASH); the first-line approach remains lifestyle changes (diet and physical exercise). The high risk of complications associated with NAFLD/NASH, such as cardiovascular diseases and the incidence of hepatocellular carcinoma, make it necessary to apply appropriate medications to stop the progression of the disease. Considering the pathogenetic pathways known today, several therapeutic targets have been proposed, including some that look very promising; still, innovative pharmacological strategies are absolutely needed. Below, we report the real efficacies of fatty liver therapies and the most recent studies investigating this field.

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a chronic hepatic disease that has swollen to epidemic proportions, affecting up to 95 million adults in the United States alone. The prevalence of NAFLD among children is constantly increasing, affecting up to 70%-80% of obese children[1]. NAFLD encompasses a spectrum of hepatic pathologies, ranging from simple steatosis to non-alcoholic steatohepatitis (NASH), cirrhosis, hepatocellular carcinoma (HCC) and end-stage liver disease[2]. This condition could be considered the hepatic manifestation of the metabolic syndrome[3] or the final expression of such unhealthy behaviors as excess calorie intake and physical inactivity[4,5]. Insulin resistance and metabolic syndrome have been implicated both in the pathogenesis and disease progression of NAFLD[6,7], causing, among other symptoms, increased free fatty acid influx to the liver, oxidative stress, mitochondrial toxicity, deregulation of adipokines and subsequently inflammation and fibrosis[8,9]. Despite the abundance of clinical trials, NAFLD therapy remains a challenge for the scientific community, and there are no licensed therapies for NAFLD. Moreover, lifestyle modifications, such as diet and physical exercise, may be proven to be effective, but they are challenging to implement[10,11].

This study is focused on the cornerstones of NAFLD treatments and on potential innovative therapies. A computerized advanced search was performed in PubMed (Public/Publisher MEDLINE) and in www.clinicaltrial.gov using a combination of terminology and methodology search filters.

Bullet points

(1) NAFLD has reached endemic proportions; and (2) NAFLD therapy remains a challenge.

TREATMENT STRATEGIES

To date, therapeutic trials for NAFLD have aimed at the weakening of all pathogenic hits, striving for decreases in steatosis, insulin resistance (IR), oxidative stress, inflammation and fibrosis. The therapeutic perspectives for patients with NAFLD have only partly changed in the last few years. In fact, in recent years, visceral adipose tissue and IR have taken leading roles in NAFLD pathogenesis, such that the current therapeutic approaches should shift to establish lasting reductions in visceral obesity the overflow of free fatty acids (FFAs) and to maintain valid improvements in IR. Despite these new findings, approaches to the management of NAFLD can be so far divided into three groups: lifestyle changes (diet and/or physical exercise), medications and surgical interventions. However, as suggested by the practice guidelines from the American Gastroenterological Association, a different perspective on the therapeutic management of NAFLD is emerging[12]. This perspective considers the initial selection of the target as a choice between two different approaches: "treat the patient" by lifestyle changes (*i.e.*, nutritional counseling and physical activity) and/or weight loss (*i.e.*, bariatric surgery and anti-obesity drugs) or "treat the liver" using different drugs to protect from liver damage [*i.e.*, insulin sensitizer, anti-oxidants, anti-tumor necrosis factor (TNF)-α, *etc.*]. Moreover, the treatment of NAFLD is challenging; considering the magnitude of the problem, we will attempt, in this review, (1) to provide a wide perspective on the real efficacies of different available medical therapies; and (2) to focus on the most recent findings in existing therapies, as well as on new horizons of research and the major emerging drugs in fatty liver disease.

Main treatments for fatty liver disease

The main procedures suggested for the treatment of NAFLD are shown in Table 1.

Bullet points

(1) The therapeutic management targets of NAFLD are evolving; "treat the patient" and "treat the liver" should be both considered; and (2) At present, there are three approaches to NAFLD treatment: lifestyle changes (diet and/or physical exercise), medications and surgical interventions.

LIFESTYLE INTERVENTION

Weight loss, diet and physical exercise

Regardless of the limited data and their imperfect quality of analysis, results from long-term, randomized intervention studies show sufficient evidence to support the use of diet and exercise for the management of NAFLD and the associated co-morbidities, such as metabolic syndrome. Diet and exercise are rightfully considered the first line of treatment for patients with NAFLD. Weight loss, achieved either by diet or exercise, has been documented to be efficient in decreasing the liver content of triglycerides (TGs) in patients with simple steatosis. These results have not been well documented for subjects with NASH[13,14]. In fact, a weight loss of 5%-10% from baseline has been shown in different studies to reduce significantly hepatic steatosis, but its effects on inflammation and fibrosis have not been sufficiently evaluated[15,16]. Furthermore, weight loss equal to or greater than 7% from baseline was associated with a significant decrease in steatosis, lobular inflammation and ballooning, but not in fibrosis. The guidelines used in Asia actually recommend that a reduction of at least 3%-5% of the body weight is necessary to ameliorate steatosis, but a greater weight loss (up to 10%) may be

Table 1 Main treatments and emerging drugs for patients with non-alcoholic fatty liver disease

Lifestyle intervention	Anti-obesity drugs	Glucose control	Lipid-lowering drugs	Cytoprotective agents	Other drugs	Emerging drugs and new horizons
Diet	Orlistat	Insulin-sensitizing agents	Statins, fibrate and ezetimibe	Ursodeoxycholic acid	Probiotics	Inhibitors of caspases
Weight loss	Cannabinoid agonists	New anti-diabetic drugs	Probucol	Anti-oxidants	Anti-TNF-α monoclonal antibodies	Adenosine system drugs
Physical exercise		Renal glucose transporters blockers		Herbal products	Anti-hypertensive drugs	PPAR agonists
Surgery (not treated)					Pentoxifylline	Peripheral cannabinoid agonists
					Iron depletion	Farnesoid X receptor agonists
						Thyroid hormone analogues

TNF: Tumor necrosis factor; PPAR: Peroxisome proliferator-activated receptors.

needed to improve necro-inflammation[17]. A recent meta-analysis by Musso *et al*[18] demonstrated the real efficacy of weight loss in improving the histological disease severity of NASH. The same study showed that the majority of the patients are unable to reach their weight goal with lifestyle interventions alone. In fact, the effects of weight loss are inconstant due to the limited compliance of patients to diet and the exercise[19]. An additional limitation to this therapeutic field is the "quality of diet". In fact, the exact types of dietary intervention or physical exercise that are really effective for patients with NASH have not been well defined. The accurate composition of an alimentary diet for patients affected by NAFLD is unidentified, but the importance of consuming a correct diet in this disease has certainly been proven by improvements in the peripheral and hepatic insulin sensitivity, the reduction of hepatic FFAs storage and the amelioration of adipose tissue inflammation[20]. Recent data have markedly shown that energy intake is significantly higher in NAFLD patients than in individuals without this entity[21]. Food quality, in fact, has been shown to have a great relevance in NAFLD pathogenesis because patients affected by NAFLD have high intakes of saturated fatty acids (SFAs) and cholesterol, an elevated consumption of drinks containing fructose and a low consumption of vitamins A and E[22-24]. Recent studies on humans have demonstrated that a high level of fructose in the diet may induce NAFLD and the metabolic syndrome through different mechanisms, such as bacterial translocation from gut to liver and the formation of advanced glycation end-products[25]. Therefore, the quality of calories consumed must be strictly evaluated. Lipid-lowering therapies are safe when they are gradually implemented. Furthermore, rapid weight loss in obese patients is widely known to potentially worsen fibrosis[26]; therefore, a "qualitative rather than quantitative" weight loss must be considered as the cornerstone for the treatment of hepatic steatosis, although this goal is no less difficult to pursue. In fact, the main therapeutic failures seen using this strategy are linked to difficulties in maintaining weight for a prolonged time course. In conclusion, a pragmatic approach may be to recommend a balanced, reduced-calorie diet. Saturated fats and high glycemic foods, including simple sugars, refined grains, rice, and potatoes, should be limited or avoided in favor of whole grains, legumes, and low glycemic index fruits and vegetables. A low-carbohydrate diet reduces glucose-stimulated insulin secretion, reducing hepatic lipid synthesis and storage. Saturated fatty acids stimulate glucose-dependent insulin secretion and promote chronic hyperinsulinemia; therefore, their intake should be limited. Conversely, the consumption of polyunsaturated fats (PUFAs) from fish and flax seed oils has beneficial effects on insulin sensitivity and promotes the anti-inflammatory prostaglandin metabolism[27]. An excess of lipids in the diet, especially saturated fatty acids, is well known to be one of the most important risk factors for NAFLD onset and development[28]. Because of these findings, the over-ingestion of cholesterol has always been regarded as a trigger of NAFLD[29,30]. Investigations regarding cholesterol ingestion in NAFLD patients have shown contrasting results; several studies have reported no significant differences in the cholesterol intake levels between NAFLD patients and healthy subjects[31,32]. A dietary record of differences among obese and non-obese NAFLD patients found that cholesterol consumption was significantly greater in NAFLD patients than in healthy controls but that non-obese NAFLD patients actually ingested more cholesterol than obese NAFLD patients[33]. Physical activity, either aerobic or anaerobic, has been proven to ameliorate the different conditions linked to metabolic syndrome by improving cardiorespiratory fitness, lowering lipids accumulations and facilitating the maintenance of weight loss. However, the difficult-to-maintain cornerstone of this treatment is motivating sedentary individuals to practice physical exercise. This therapeutic target can be carried out efficiently only through structured programs following cognitive-behavior therapy[34]. Weight loss generally reduces hepatic steatosis, which is achieved either by a hypocaloric diet alone or in conjunction with increased physical activity. Physical exercise reduces hyperinsulinemia and increases insulin sensitivity and substrate oxidation independently from its modifications on body weight[35]. Exercise alone in adults with NAFLD may reduce hepatic steatosis, but its ability to improve other aspects of liver histology remains unknown[12]. In fact, a recent review by Finelli *et*

al[35] concluded that more rigorous, controlled studies of longer durations and better defined histo-pathological end-points are needed to compare exercise alone with other treatments before better, evidence-based physical activity modification guidelines can be established. Several questions remain unanswered[35].

New findings in lifestyle approaches: An appropriate intake of carbohydrates and fiber with the diet can prevent and ameliorate NAFLD and its complications. A recent review focused on the therapeutic effects of diets rich in whole grains on many of the co-morbidities associated with NAFLD, including NASH[36]. In fact, whole grains contain higher amounts of compounds that may help to reduce liver fat accumulation and to protect against inflammation, avoiding progression to NASH. Several meta-analyses had studied the benefits of whole grains against the risk of developing T2DM and obesity. These grains, in addition to carbohydrates, contain antioxidative vitamins, minerals, and dietary fiber, which combine into several possible beneficial mechanisms beyond better nutrient intake. For example, the reduction of energy intake and subsequent changes to and stimulation of the gut microbiota can lead to the increased production of short-chain fatty acids. A recent investigation on dietary fat intake clearly demonstrated the effect of dietary fat content on liver fat accumulation in an older population. Patients affected by NAFLD were randomly allocated onto an iso-energetic low-fat/low-saturated fat (LSAT)/low-glycemic index (GI) diet (LSAT: 23% fat/7% saturated fat/GI < 55) or a high-fat/high-saturated fat (HSAT) /high-GI diet (HSAT: 43% fat/24% saturated fat/GI > 70). The liver fat was quantified by magnetic resonance spectroscopy before and after 4 wk on the LSAT and HSAT diets. This study showed that the LSAT *vs* the HSAT diet was a predictor of changes in lipid parameters, but not in liver fat. They concluded that patients in the LSAT, but not those in the HSAT, group showed significant reductions in liver fat[37].

Bullet points

(1) Weight loss is an efficient means of ameliorating hepatic steatosis, but its effect on inflammation or fibrosis has not been sufficiently evaluated; (2) Compliance to diet and the quality of diet must be carefully evaluated in each patient; (3) Saturated fats and high glycemic foods, including simple sugars, refined grains, rice, and potatoes, should be limited or avoided in favor of whole grains, legumes, PUFAs and low glycemic index fruits and vegetables; and (4) Physical activity ameliorates hepatic steatosis and aids weight loss maintenance, but its efficacy for fibrosis and necroinflammation has not been well investigated.

Anti-obesity drugs

Orlistat: Orlistat has been widely proposed in the last years as a weight-loss aid because of its properties as inhibitor of fat enteric absorption. Orlistat has been tested in NASH patients, but the results have shown no histological improvement[38,39]. In a recent randomized controlled study (RCT) conducted by Harrison and his group, orlistat was demonstrated to be safe and well tolerated with a mean of 10 kg weight loss after 6 mo of treatment and improved alanine aminotransferase (ALT) levels. However, the use of orlistat did not add any further cardio-metabolic changes or histological reductions in steatosis over lifestyle modification alone.

Cannabinoid agonists: Over the last decade, the endocannabinoid system has emerged as a pivotal mediator of acute and chronic liver injury, with the description of the role of CB1 and CB2 receptors and their endogenous lipidic ligands in various aspects of liver pathophysiology. CB1 receptors expressed in hepatocytes and hepatic myofibroblasts contribute to high fat storage and alcohol-induced steatosis, liver regeneration, and fibrogenesis. The steatogenic properties of CB1 result from the hepatocyte activation of lipogenesis, reduction of fatty acid oxidation, and decreased release of TG-rich VLDL, combined with the CB1-dependent release of free fatty acids from the adipose tissue. CB1 also activates hepatocyte proliferation and promotes fibrogenesis by enhancing hepatic myofibroblast survival. There is accumulating evidence on the role CB1 as a key mediator of insulin resistance, enhancing its role in the development of liver steatosis and steatohepatitis[40]. In contrast to CB1, the role of CB2 receptors in the development of fatty liver has not been well investigated. Animal studies have demonstrated that CB2 receptor expression receives a strong induction in adipose tissue that correlated with increased fat inflammation[41]. These results have been supported by the finding that the administration of CB2 agonists enhanced liver TG accumulation, IR and fat inflammation in wild type mice[42]. On the other hand, there is some evidence of a potentially anti-fibrotic effect of CB2 receptor activation distinct from that of CB1, which has been classified as pro-fibrogenic receptor[43]. CB1 receptor antagonism and CB2 receptor agonism have been identified as promising therapeutic strategies for the management of liver diseases. The widely investigated CB1-antagonist rimonabant, which was initially approved for the management of overweight, liver steatosis and related cardio-metabolic risks[44], was withdrawn because of its alarming rate of adverse effects on mood, primarily psychiatric in nature due to its concentration in the brain. Linked to this adverse effect are peripherally-restricted CB1 antagonists with limited brain concentrations, which have been proposed and tested with promising results.

Bullet points

(1) Orlistat has been tested in NAFLD and NASH patients and been found to be always lacking in simple steatosis reductions over lifestyle modification alone or histological improvements in necro-inflammation; (2) The endocannabinoid CB1 receptors expressed in hepatocytes and hepatic myofibroblasts contribute to high fat storage,

alcohol-induced steatosis, fibrogenesis and insulin resistance; (3) Endocannabinoid CB2 receptors have not been as widely investigated as CB1 receptors; (4) CB1 receptor antagonism and CB2 receptor agonism have been identified as promising therapeutic strategies for the management of liver diseases; (5) Rimonabant (CB1-antagonist), which was initially approved for the management of overweight, liver steatosis and related cardio-metabolic risks, was withdrawn due to an alarming rate of adverse effects on mood, primarily psychiatric in nature due to its concentration in the brain; and (6) Linked to this adverse effect, peripherally-restricted CB1 antagonists with a limited brain concentration have been proposed and tested to promising results.

INSULIN-SENSITIZING DRUGS

Metformin and glitazones

Considering the close relationship between IR and the pathogenesis of fatty liver disease, insulin sensitizers could be regarded as the treatment of choice. Metformin and glitazones (TZDs) are the most popular drugs tested against NAFLD/NASH.

Metformin improves IR by decreasing hepatic glucose production and increasing skeletal muscle glucose uptake. This drug also reduces the hepatic expression of TNF-α, a mediator of hepatic insulin resistance and necro-inflammation; increases FFA oxidation; and suppresses lipogenesis through AMP-kinase activation. Metformin has been demonstrated to be safe and well tolerated in different studies, with poor cases of lactic acidosis and gastrointestinal intolerance emerging as the most common side effects but not generally requiring discontinuation of the therapy[45]. Despite these positive results regarding tolerance, further well-designed studies are needed to elucidate the significance of metformin treatment for NAFLD. In fact, not all studies have reported the same results for both serum liver enzymes and liver histology. The TONIC trial, which was the most recent randomized study of metformin, compared metformin with vitamin E and placebo in children and adolescents[46]. Again, the results were not satisfactory; in fact, there was no significant reduction in transaminases compared with placebo in the metformin group, and there was no significant change in histology, except for hepatocellular ballooning. Based on the results of these studies and others, metformin is not recommended as a specific treatment for liver disease in adults with NASH[47,48].

The TZDs are agonists of the peroxisome proliferator-activated receptors (PPAR)-γ. PPAR-γ, which belongs to the nuclear hormone receptor family, is predominantly expressed in adipose tissues and plays a key role in adipogenesis and glucose homeostasis[49]. After the withdrawal of troglitazone and rosiglitazone for hepatic and cardiac toxicity, studies testing the efficacy of TDZs in the fatty liver have focused on a new molecule, pioglitazone. A large multicenter RCT study, named the PIVENS study, showed that, in non-diabetic patients with NASH, pioglitazone ameliorated the histological features of NASH but not simple steatosis. Moreover, pioglitazone was associated with weight gain[50]. Another RCT study by Belfort *et al*[51] was conducted on NASH patients with impaired glucose tolerance or T2DM. Significant weight gain was confirmed, accompanied by an improvement in the aminotransferase levels, steatosis and inflammation. Conversely, discontinuing TZD therapy has been shown to cause an immediate NASH recurrence. Long-term use is required for the achievement of treatment results; however, the duration can cause medical complications, such as edema, congestive heart failure, osteoporosis and weight gain[52]. Overall, pioglitazone is commonly used as a treatment for NASH, as indicated by AASLD guidelines[48]. However, the long term safety and efficacy of pioglitazone in patients with NASH have not been established.

New anti-diabetic medications

Glucagon-like peptide-1 (GLP-1) is an incretin secreted by the small intestine in response to food intake that improves glucose homeostasis *via* its glucose-dependent stimulation of insulin secretion, inhibition of postprandial glucagon secretion and delayed gastric emptying. Once secreted, GLP-1 has a short half-life due to its rapid degradation by dipeptidyl peptidase 4 (DPP-4)[53]. DPP IV inhibitors and GLP-1 analogues are novel agents that can overcome rapid degradation and have been used successfully in the treatment of T2DM[30f-30h]. The rationale of their use is to obtain indirect improvements of the fatty liver secondary to optimizing glycemic control by weight loss, hepatic insulin sensitivity improvement, hepatic fatty acid oxidation improvement, and inhibition of the fibroblast growth factor 21. Synthetic GLP-1 agonists[exenatide (Amylin Pharmaceuticals, Inc., San Diego, CA, United States) and liraglutide (Novo Nordisk A/S, Bagsvaerd, Denmark)] are available for the treatment of T2DM[54-56]. Four DPP-4 inhibitors are currently on the market: linagliptin (Boehringer Ingelheim International GmbH, Ingelheim, Germany), saxagliptin (Bristol-Myers Squibb, Princeton, NJ, United States), sitagliptin (Merck & Co., Inc., Whitehouse Station, NJ, United States), and vildagliptin (Novartis, Basel, Switzerland; approved in various countries in Europe, Asia Pacific, Africa and Latin America). Several human and animal studies have demonstrated the therapeutic effects of GLP-1 receptor agonists and DPP-4 inhibitors in preventing and improving NAFLD. The molecules most tested in fatty liver disease were exenatide and sitagliptin. In a recent study by Svegliati-Baroni *et al*[57], exenatide, a glucagon-like peptide-1 analogue, improved fatty acid β-oxidation and insulin sensitivity in rats with NASH resulting from 3 mo of the high-fat diet. Exenatide, when compared with metformin, is better able to control blood glucose values, improving body weight and ameliorating hepatic enzymes in patients with NAFLD concomitant with T2DM[58]. Sitagliptin showed a decrease in the liver TG content, the expression of lipogenesis genes and gluconeogenesis in wild type mice[59]. A recent study by

Iwasaki *et al*[60] showed that treatment with sitagliptin improved glycemic control and liver tests. These new anti-diabetic medications may have significant advantages for the management of NASH in terms of efficacy and tolerability[61]. These promising results should guide future RCTs to establish the role of these agents in diabetic and non-diabetic NAFLD/NASH patients.

Renal control of hyperglycemia

Recent findings investigating the renal sodium glucose transporter-2 (SGLT-2) in the kidney have shown that drugs acting by blocking glucose reabsorption and subsequently increasing glucosuria significantly improve hyperglycemia, insulin sensitivity and adipose tissue inflammation. The most studied drug blocking the SGLT-2 transporter is sergliflozinetabonate. In mice exhibiting diabetes and fatty liver, treatment with sergliflozinetabonate improved hyperglycemia, fatty liver and pancreatic beta-cell abnormalities[62]. Future studies on the role of SGLT-2 inhibitors in fatty liver disease on animals and humans are awaited.

Bullet points

(1) Metformin and TZDs are the most popular drugs tested in NAFLD/NASH; (2) At this time, metformin is not recommended as specific treatment for NAFLD/NASH; (3) Pioglitazone is commonly used as a treatment for NASH, as indicated by the AASLD guidelines. However, the long-term safety and efficacy of pioglitazone in patients with NASH are not well established; (5) New anti-diabetic medications, such as GLP-1 receptor agonists and DPP-4 inhibitors, have significant advantages in the management of NASH in terms of efficacy and tolerability; and (6) Drugs acting by blocking glucose reabsorption and subsequently increasing glucosuria significantly improve hyperglycemia, insulin sensitivity and adipose tissue inflammation. The most studied drug blocking the SGLT-2 transporter has been sergliflozinetabonate. More studies are awaited.

LIPID-LOWERING DRUGS

Lipid-lowering drugs have been widely studied for the treatment of fatty liver because of the simple rationale of preventing the accumulation of FFAs and TGs in the hepatocytes. Between 1980 and December 2012, a report of human studies, including pilot, prospective, preliminary, and post hoc analysis studies, from online databases showed that lipid-lowering agents, such as statins, fibrates and ezetimibe, used alone or in combination with cytoprotective agents, are successful and safe in patients with NAFLD/NASH. Moreover, these agents have shown great results on hepatic histology by inducing a reduction in hepatic steatosis[63]. Despite these excellent analytic results, the report concludes that well-designed RCTs of adequate size and duration with well-defined histological endpoints are needed to establish a suitable lipid-lowering treatment for hyperlipidemic patients with NAFLD/NASH and for non-hyperlipidemic patients with NAFLD/NASH with a high risk for cardiovascular disease.

Probucol

Probucol is an anti-hyperlipidemic agent with powerful antioxidant activity that prevents lipid oxidation. This drug has shown promising results based on its significant reductions in aminotransferase levels and improvements in liver histology among a small observational study of 8 patients[64]. An RCT showed that probucol was significantly effective in decreasing the ALT levels in patients with NASH[65]. However, probucol concomitantly decreased HDL-cholesterol levels, which are causes of concern in patients with coronary artery disease. There are no recent studies that have supported probucol as a promising new drug.

Bullet points

(1) Lipid-lowering agents, such as statins, fibrates and ezetimibe, used alone or in combination with cytoprotective agents, are successful and safe in patients with NAFLD/NASH. However, this encouraging report concludes with a statement that well-designed RCTs are necessary to establish a suitable treatment; and (2) Probucol is an anti-hyperlipidemic agent with powerful antioxidant activity; however, there are no recent studies that have supported probucol as a promising new drug.

CYTOPROTECTIVE AGENTS (URSODEOXYCHOLIC ACID, ANTI-OXIDANTS AND HERBAL ANTI-OXIDANTS PRODUCTS)

Ursodeoxycholic acid

The rational of ursodeoxycholic acid (UDCA) therapy in fatty livers derives from the evidence of its role in decreasing cholesterol secretions into bile. Moreover, different studies have shown its hepato-protective effects by acting on many pathways leading to counteract the pathogenic mechanisms involved in the transition from steatosis to steatohepatitis[66]. Early clinical studies have suggested a potentially beneficial effect in both NAFLD and NASH. Still, only few data on the efficacy of UDCA are available[67]. At present, there are insufficient data to either support or refuse the use of UDCA[68]. In fact, two large RCT showed that, after 2 years of treatment with UDCA, there is no significant improvement in the liver histology of patients affected by NASH[69,70]. AASLD Guidelines do not recommend UDCA for the treatment of patients with NAFLD or NASH[48].

Anti-oxidants and herbal anti-oxidant products

Antioxidants have therapeutic potential because fatty acid oxidation produces reactive oxygen species (ROS), causing direct cellular damage and activating pro-inflamma-

tory cytokines. This is considered the key mechanism for the progression of the disease from simple steatosis to NASH, cirrhosis and finally HCC. The biological effects of oxidative stress are neutralized *in vivo* by anti-oxidative defense mechanisms that include mainly vitamins (C and E), enzymes with antioxidant activity, carotenoids and glutathione (GSH). Vitamin E is certainly the substance that has been the most widely investigated for the treatment of NAFLD and NASH in both pediatric and adult populations[46,71-73]. Vitamin E has been shown to be associated with decreased aminotransferase levels and improvements in steatosis, inflammation, ballooning and resolution of steatohepatitis in adults with NASH. Similar results were obtained in a pediatric population (the TONIC study)[46]. However, the role of vitamin E in liver fibrosis is uncertain[71]. Vitamin E is recommended as a first-line treatment for non-diabetic patients with histologically confirmed NASH according to the actual NAFLD AASLD guidelines. On the other hand, vitamin E is not recommended for NASH patients with diabetes[48]. The absolute indication for recommendation of the use of vitamin E as first-line treatment for non-diabetic patients with biopsy-proven NASH derives from the PIVENS study, which is the largest clinical trial reported to date[73] to clearly show the benefits of vitamin E in patients with biopsy-proven NASH. A new Japanese study with vitamin E at a dose of 300 mg/d in patients with biopsy-proven NASH has recently confirmed the results of a previous study showing that long-term treatment (more than 2 years) seems to be more effective than placebo in ameliorating histological fibrosis and normalizing the serum values of transaminases[50,74]. Despite these excellent results, an important concern with the long-term use of vitamin E remains the probability of an increase in all-cause mortality, as detected by recent meta-analysis[75,76]. Natural substances deriving from herbal products comprise a good field of research for the prevention and treatment of fatty liver disease and the associated conditions. We have recently published a review on the role of some natural antioxidants deriving from herbal products in chronic liver diseases[77]. We report below our findings about the beneficial effects of some "natural" antioxidants that have already been known for some time and on others that are less known. Silybum marianum, also known as milk thistle, is a member of Asteraceae family and is well recognized as a hepatoprotective herbal medicine. Silymarin is a lipophilic extract from the milk thistle seeds that is composed of three isomers of flavonolignans (silybin, silydianin, and silychristin) and two flavonoids (taxifolin and quercetin). Silymarin possesses various pharmacological activities, including hepatoprotective, antioxidant, anti-inflammatory, anticancer, and cardioprotective effects. Silybum marianum is the best-researched plant for the treatment of liver diseases. Silymarin has been shown to have a variety of anti-inflammatory effects on the liver, including mast cell stabilization, inhibition of neutrophil migration and Kupffer cell inhibition[78]. Silymarin is commonly prescribed in cases of cirrhosis or viral hepatitis. Assuming that oxidative stress leads to chronic liver damage, Loguercio *et al*[79] conducted a study about the antioxidant activity of silybin conjugated with vitamin E and phospholipids. Our data suggest that silybin conjugated with vitamin E and phospholipids could be used as a complementary approach in the treatment of patients with chronic liver damage. Quercetin is among the major flavonoids, which comprise a class of naturally occurring polyphenolic compounds that is ubiquitously present in photosynthesizing cells. The optimal intake of flavones and flavonols is determined as 23-24 mg/d, and quercetin, the main flavonol present in our diet, represents 70% of this intake. Quercetin is found in fruits (apples) and vegetables, especially onions[80]. The hepatic response to chronic noxious stimuli may lead to liver fibrosis and to pre-neoplastic cirrhotic liver. Fibrogenic cells become activated in response to a variety of cytokines, growth factors, and inflammatory mediators. The involvement of members of the epidermal growth factor family in this process has been suggested. Amphiregulin is an epidermal growth factor receptor (EGFR) ligand that is specifically induced upon liver injury. A recent study investigated the effects of quercetin on amphiregulin/EGFR signalling and on the activation of the downstream pathways leading to cell growth and found that quercetin ameliorated activation of survival pathways and downregulated the expression of genes related to inflammation and precancerous conditions. Betaine is a naturally occurring dietary compound that is also synthesized *in vivo* from choline. *In vivo*, betaine acts as a methyl donor for the conversion of homocysteine to methionine and as an osmolyte. The role of betaine in the treatment of NASH has been evaluated in human studies. The oral administration of betaine glucuronate in NASH patients for 8 wk reduced hepatic steatosis by 25% and hepatomegaly by 8% and significantly attenuated the serum concentrations of aspartate aminotransferase (AST), ALT and γ-glutamyltransferase. Similarly, a marked improvement in the degree of steatosis, necro-inflammatory grade and stage of fibrosis was obtained after treatment with betaine[81]. Betaine treatment reversed the inhibition of hepatic insulin signaling in mHF and in insulin-resistant HepG2 cells, including the normalization of insulin receptor substrate 1 (IRS1) phosphorylation and of signaling pathways for gluconeogenesis and glycogen synthesis. Moreover, betaine supplementation alleviated the hepatic pathological changes that were concomitant with the reductions in insulin resistance (as shown by the improved homeostasis model assessment of basal insulin resistance values and glucose tolerance test) and corrected abnormal adipokine productions (adiponectin, resistin, and leptin). Specifically, betaine supplementation enhanced insulin sensitivity in the adipose tissue, as shown by the improved extracellular signal-regulated kinases 1/2 and protein kinase B activations. In adipocytes freshly isolated from mice fed a high-fat diet, pretreatment with betaine enhanced the insulin signaling pathways and improved adipokine productions.

Table 2 Characteristics of the included studies within the meta-analysis of Ma *et al*[88]

Ref.	Sample size	Randomization	Blinding	Diagnostic method	Intervention	Duration	Follow-up
Aller *et al*[87]	28 (14/14)	Table of numbers	Double-blind	Histological	*Lactobacillus bulgaricus* and Streptococcus thermophilus *vs* placebo	3 mo	Yes
Vajro *et al*[90]	20 (10/10)	Yes	Double-blind	Radiological	Lactobacillus GG *vs* placebo	8 wk	Yes
Malaguarnera *et al*[91]	66 (34/32)	Computer generated	Double-blind	Histological	Bifidobacteriumlongum + Fos *vs* placebo	24 wk	Yes
Wong *et al*[92]	20 (10/10)	Computer generated	Double-blind	Histological	Lepicol probiotic and prebiotic formula *vs* nothing	6 mo	Yes

Further investigation using whole liver tissues revealed that betaine supplementation alleviated high-fat diet-induced endoplasmic reticulum stress response in the adipose tissue, as shown by attenuated glucose-regulated protein 78/C/EBP homologous protein (CHOP) protein abundance and c-Jun NH2-terminal kinase activation[82]. Song *et al*[83] showed that betaine significantly attenuated the hepatic steatosis induced by high-sucrose diet (animal model). This change was associated with increased activation of hepatic AMP-activated protein kinase (AMPK) and attenuated lipogenic capability (enzyme activities and gene expression) in the liver.

Bullet points

(1) The AASLD Guidelines do not recommend UDCA for the treatment of patients with NAFLD or NASH; (2) Vitamin E is recommended as a first-line treatment for non-diabetic patients with histologically confirmed NASH in the actual NAFLD AASLD guidelines. On the other hand, vitamin E is not recommended for NASH patients with diabetes; (3) Despite these excellent results, the important concern with the long-term use of vitamin E lingers, feeding off the probability of an increase in all-cause mortality detected by a recent meta-analysis; and (4) Natural substances deriving from herbal products, such as Silymarin, Betaine, and Quercetin, remain a good field of research for the prevention and treatment of fatty liver disease and its associated conditions.

OTHER DRUGS

Probiotics

NAFLD and its strong association with microbiota have elicited interest in the underlying mechanisms of these pathologies. Experimental models have highlighted several mechanisms connecting the microbiota to the development of liver dysfunction in NASH, such as increased energy-harvesting from the diet, small intestine bacterial overgrowth, modulation of the intestinal barrier by glucagon-like peptide-2 secretions, and activation of innate immunity through the lipopolysaccharide-CD14 axis caused by obesity-induced leptin[84]. The liver is continually exposed to gut-derived factors, including bacteria and bacterial components, as the portal vein is the direct venous outflow of the intestine. The liver is an important site for bacterial phagocytosis and clearance because it contains the largest population of tissue macrophages. Activated Kupffer cells, the resident macrophages of the liver, when exposed to pro-inflammatory mediators, such as LPS, membrane components of Gram-negative bacteria, or other bacterial products, are a major source of inflammatory mediators, including pro-inflammatory cytokines, chemokines, and reactive oxygen/nitrogen species, which contribute to liver injury. Lastly, a recent study in humans demonstrated that NAFLD is associated with the increased gut permeability caused by disruptions in the intercellular tight junctions of the intestine and that this condition may play an important role in the pathogenesis of hepatic fat deposition[85]. Probiotics and prebiotics are important mediators of diet-induced metabolic disturbances in NAFLD. The manipulation of the microbiota through probiotics, prebiotics, and antibiotics has generated encouraging results for the treatment of obesity, diabetes, and NASH, although data in humans are scarce[84]. We have complied a table to summarize one of the most recent meta-analyses (Table 2). The use of probiotic, prebiotic and symbiotic bacteria is recommended, since these bacteria have the capacity to modulate microflora overpopulations and their consequent effects on the liver. In spite of the presence of various experimental studies in this field and the known positive effects of probiotisc on induced liver steatosis, the use of this path as a strategy for treating or even preventing NAFLD is still complicated. Most of these experiments were performed on the effects of VSL#3 administration, which itself contains a various species such as *Streptococcus thermophilus, Bifidobacterium berve, Bifidobacterium longum, Bifidobacterium infanti, Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus casei*, and *Lactobacillus bulgaricus*; the determination of the effect of each species is unclear. Loguercio *et al*[86] showed that probiotics may reduce NAFLD liver injury and may improve liver enzymes. Probiotics can inhibit the proliferation of harmful bacteria, reduce small intestinal bacterial overgrowth (SIBO), restore gastrointestinal barrier function and modulate the immune system, all of which contribute to the improvement of NAFLD. The species of probiotics used in these studies have varied. Another recent study using a randomized double blind clinical trial evaluated the effects of a different probiotic. This study evaluated the effects of Lactobacillus bulgarius and Streptococcus thermophilus (1 tablet/d) in 28 NAFLD patients over a 3-mo period. The results showed decreased ALT, AST and γ-glutamyltransferase levels[87]. Probiotic therapies can reduce liver aminotransferase,

serum cholesterol and TNF-α levels while improving insulin resistance in patients with NAFLD. A recent meta-analysis[88] included four RCTs that studied probiotics, including lactobacillus, bifidobacterium and streptococcus. Bifidobacteria produce a range of beneficial effects on host health. Lactobacilli and streptococcus are also beneficial, although they are present at much lower levels in the human colon. Probiotics have been shown to enhance the barrier function of epithelial cells and to decrease intestinal permeability and endotoxemia in patients with liver disease. At the same time, probiotics can also influence host metabolism in several other ways, such as the regulation of energy extraction from nutrients and the modulation of genes involved in substrate metabolism. A prebiotic is a non-digestible food ingredient. The general properties of prebiotics include the capacity to influence the growth, activity and metabolites of probiotics.

Fructo-oligosaccharides are now becoming increasingly popular due to their prebiotic effects and can be fermented by bifidobacteria and lactobacilli[89]. Fructo-oligosaccharides can lead to the emergence of bifidobacteria as the dominant species in the large bowel[90] and may help to control or reduce the growth of harmful bacteria[91]. It is well known that liver histology is the gold standard for NAFLD/NASH. Therefore, approving the use of these bacteria as a treatment protocol requires several large-scale clinical trials. The existing data are difficult to reconcile, given the use of different strains, dosages and durations of treatment[92].

Anti-TNF-α monoclonal antibodies

The balance between the pro- and anti-inflammatory actions of cytokines/adipocytokines appears to play a key role in hepatic and systemic insulin action, and they are supposed to have important functions in the development of NAFLD. In the literature, there are few works (in animal models) that have studied the actual effects of anti-TNF-α therapy in the treatment of NAFLD. These studies indicate that anti-TNF-α antibodies are effective against necrosis, inflammation and fibrosis in the experimental model of non-alcoholic steatohepatitis[93,94]. More studies on animal models are needed before testing these molecules on humans.

Anti-hypertensive drugs

Recent and past studies have focused on the effects of some antihypertensive drugs, especially those inhibiting the renin-angiotensin system, on hepatic fibrosis rather than NAFLD[95]. Recent studies have in fact affirmed a potentially beneficial effect of Valsartan on liver disease[96,97]. Qiang *et al*[96] investigated the effect of Valsartan on the pathological progression of hepatic fibrosis in rats with T2DM. An animal model of hepatic fibrosis with T2DM was developed using a high-sucrose, high-fat diet and low-dose streptozotocin. Valsartan (15 mg/kg per day, *i.g.*) was orally administered for four months. The results showed that valsartan significantly alleviated the lesions from hepatic steatosis and hepatic fibrosis. Immuno-histochemical staining suggested that the expression of α-SMA, TGF-β1, TNF-α and MCP-1 in the liver tissue of diabetic rats was markedly reduced by valsartan. In addition, valsartan restored the injured hepatic mitochondrial respiratory function. These findings demonstrate that valsartan prevented the pathological progression of hepatic fibrosis in type 2 diabetic rats, as correlated with a reduced α-SMA, TGF-β1, TNF-α and MCP-1 expression and anti-apoptotic and mitochondria-protective potential. Previous studies have shown that the renin-angiotensin system (RAS) plays an important role in the pathogenesis of hepatic fibrosis, and blockers of the RAS may act as an anti-fibrogenic goal. However, the potential role of RAS inhibition in hepatic fibrosis remains unknown.

Pentoxifylline

Pentoxifylline (PTX) improved the histological features of NASH in a recent RCT. However, the underlying mechanism responsible for the beneficial effects of PTX on NASH remains unidentified. A key role of lipid oxidation in the pathogenesis and progression of NASH has been established. PTX is known to decrease free-radical-mediated oxidative stress and to inhibit lipid oxidation. Therapy with PTX compared to placebo was associated with a significant reduction in the oxidized fatty acids. This novel evidence supports the fact that the beneficial effects of PTX in patients with NASH are likely to be partly mediated through decreasing lipid oxidation, largely free-radical-mediated lipid oxidation. No association was demonstrated between therapy with PTX and changes in insulin sensitivity, as measured by frequently sampled intra-venous glucose tolerance testing, on levels of TNF-α[98]. This study demonstrated that therapy with PTX in NASH results in decreased oxidized lipid products of Linoleic acid (LA) and Arachidonic acid (AA) compared to placebo. The decreased levels of oxidized fatty acids are shown to correlate with histological improvement (liver histopathology was evaluated before and after the intervention). The correlation between decreased oxidized lipid products and improved liver fibrosis scores is particularly noteworthy[99].

Iron depletion

Evidence has shown that increased ferritin levels are associated with the metabolic insulin resistance syndrome, as well as higher hepatic iron and fat contents. Serum hyperferritinemia and iron stores have been associated with the severity of liver damage in NAFLD, and iron depletion reduced insulin resistance and liver enzymes[100]. The elevated iron indices described in NAFLD, as well as iron reductions, have been suggested as a potential therapy. A Phase Ⅱ clinical trial registered with the US National Institute of Health valuated phlebotomy therapy in NAFLD patients, finding that iron reduction may improve liver histology. Iron reduction resulted in a significant improvement in the NAFLD activity score. Neither reductions in the individual histological features of

lobular inflammation, steatosis, and hepatocyte ballooning nor fibrosis scores achieved significance in this study. However, the effect size of phlebotomy raises questions as to whether this treatment could have sufficient clinical significance to justify a definitive Phase Ⅲ trial[101].

Bullet points

(1) The manipulation of microbiota through probiotics, prebiotics, antibiotics is providing encouraging results for the treatment of obesity, diabetes, and NAFLD/NASH, but data in humans are scarce; (2) Probiotics may reduce NAFLD liver injury and may improve liver enzymes. Most of these experiments were built around the effects of VSL#3 administration; (3) Fructo-oligosaccharides are now becoming increasingly popular due to their prebiotic effects, but the existing data are difficult to reconcile, given the use of different strains, dosages and durations of treatment; (4) Studies on an experimental model of non-alcoholic steatohepatitis indicate that anti-TNF-α antibodies are effective against liver necrosis, inflammation and fibrosis. More studies on animal models are needed before testing these molecules on humans; (5) The potential role of RAS inhibitors, such as Valsartan, in hepatic fibrosis has been poorly studied and remains largely unknown; (6) The underlying mechanism responsible for the beneficial effects of PTX in NASH remains unidentified and (7) Elevated iron indices are described in NAFLD, and so, iron reduction has been suggested as a potential therapy. The effect size of phlebotomy remains under debate.

EMERGING DRUGS AND NEW HORIZONS OF RESEARCH

Control of apoptosis and necro-inflammation: The inhibitors of caspases

The progression of steatosis to NASH and finally to cirrhosis and HCC is strictly linked to apoptosis and necro-inflammation. The signaling pathways of apoptosis lead to the activation of caspases, cellular proteases that degrade the structural proteins required for cell survival[102]. The evidence that apoptosis triggers hepatic stellate cells activation and liver fibrosis suggest that caspase inhibitors may be useful as an anti-fibrotic NASH therapy[103]. Different recent studies on animals and humans have begun to investigate this field of research. Based on the supposition that, in mice with a mutation of the leptin receptor (*db*/*db*) and on the MCD diet, hepatocyte apoptosis and hepatic necro-inflammation are reduced by the pan-caspase inhibitor VX-166[104], a recent study showed that, in mice on the MCD diet, liver histology, mainly necro-inflammation, and oxidative stress were greatly ameliorated by the hepato-specific deletion of caspase 8[105]. In humans, a double-blind, randomized phase Ⅱ study of 124 patients with biopsy-proven NASH indicated that the new drug GS-9450 specifically inhibited caspases 1, 8, and 9 and reduced serum ALT and cytokeratin-18 fragments after 4 wk of treatment[106]. In patients with chronic hepatitis C (http://www.gilead. com/pr_1414682), GS-9450 was withdrawn due to safety concerns.

Immuno-regulation and necro-inflammation: agonists and antagonists of "adenosine system"

Containing four receptors (A1, A2A, A2B, and A3), adenosine plays a pivotal role in the regulation of cell survival and tissue repairs *via* immuno-inflammatory modulation. During ethanol metabolism, adenosine is generated by the enzyme ecto-5'-nucleotidase, and adenosine receptor activation plays a critical role in the development of hepatic fibrosis[107]. In mice on the MCD diet, the agonist of the adenosine A2A receptor CGS21680 reduced inflammatory responses and consequent fibrosis without improving steatosis[108]. Unfortunately, the regulation of the adenosine system and its therapeutic targets have been investigated primarily in the context of ethanol-induced steatosis and steato-hepatitis. Moreover, differential effects of A1 and A2B have been observed in the context of alcohol-induced hepatic steatosis and lipogenesis. In fact, the blockage of A1 reduced the expression of genes involved in fatty acid synthesis, although blocking A2B did not reduce the expression of genes involved in fatty acid metabolism[107-109]. Thus, considering the heterogeneous and intricate results of the effects on cellular adenosine receptors and the concern that the majority of studies have investigated ethanol-induced injuries, the adenosine signaling system requires further investigation testing specific receptor agonists and antagonists.

PPAR alpha and delta

The different isoforms of the nuclear receptors PPARs regulate the expression of genes involved in lipid and glucose homeostasis, as well as the inflammatory and fibrotic responses of the liver. PPAR-α is implicated in the improvement of fatty acid oxidation; PPAR-γ, in the storage and deposition of TGs, anti-inflammatory signaling and improved insulin sensitivity (PPAR-γ); and PPAR-δ, in the amelioration of metabolic performance by controlling dyslipidemia and increasing fat oxidation in the musculature. Based on these hypotheses, different drugs acting on PPAR have been tested for the therapy of steatosis and steatohepatitis. The TDZs rosiglitazone and pioglitazone, PPAR-γ and PPAR-γ > α agonists, respectively, have been already addressed in this study. The compound GFT505, a combined PPAR-α/δ agonist, has been tested in two RCT showing a significant improvement in dyslipidemia and insulin resistance[110]. Currently, a phase Ⅱ-b study evaluating the efficacy and safety of GFT505 in patients with biopsy-proven NASH is ongoing, but no preliminary results have been posted[111]. In mice on a high-fat or MCD diet, three different PPAR agonists have been proven to improve the histological features of hepatic steatosis and inflammation: 1) the bezafibrate, a pan-PPAR agonist; 2) GW5051516, a PPAR-δ/β agonist; and 3) Wy14 643, a PPAR-α agonist[112,113].

Peripheral cannabinoid 1 receptor agonists

Tempering the excellent promises, concern for psychiatric

safety has ruled out the clinical development of some of the antagonists/inverse agonists that enter the brain. The use of peripherally restricted compounds with cannabinoid 1 (CB1) antagonist properties and limited brain concentrations represents a promising horizon of research in this field. A phase 1 study of a selective CB1 antagonists (cp-945598) in patients with NASH is in progress[114]. Three phase-2 studies to assess the effect of CBD on liver fat levels in subjects with fatty liver disease have been completed, and the initial results show a regression of liver TG accumulation measured by MRIMRS scanning without significant relevance[115]. Another phase 2 study assessed the action of two cannabinoids, GW 42004 and GW42003, alone or in combination among patients with T2DM; unfortunately, no significant change in the serum High Density Lipoprotein Cholesterol (HDL-C) concentration after 91 d of treatment was found[116]. CB1 receptors antagonism and CB2 receptor agonism remain promising therapeutic strategies for the management of liver diseases[117]. These hopes persist due to the positive results obtained from treating painful diabetic neuropathy and spasticity of multiple sclerosis with the use of active cannabinoid components (Sativex)[114,115].

Farnesoid X receptor agonists

Farnesoid X receptor (FXR) and TGR5, a G-protein coupled receptor, play an important role in lipid control, carbohydrate metabolism and inflammatory responses. In fact, the activation of FXR or TGR5 improves insulin sensitivity and glucose uptake in the adipose tissue, the liver and the skeletal muscle; reduces hepatic TG levels; inhibits inflammation; and ameliorates fibrosis. Different recent data have shown that biliary acids may function as signaling molecules by binding to FXR and TGR5[118-120]. Different studies have been conducted or are ongoing to test the molecules acting on these receptors in NAFLD disease. Deficiencies in the FXR receptors cause hepatic steatosis associated with inflammation and injury[121]. In mice on the MCD diet showing the hepatic histological features of steatohepatitis, the FXR agonist WAY-362450 has been shown to protect against NASH by reducing transaminase serum levels, inflammatory cell infiltration and hepatic fibrosis[122]. The confirmation of these therapeutic effects derives from the demonstration that the hepato-protection granted by WAY-362450 is not present in FXR-deficient mice. No clinical development is currently foreseen for this compound. Other compounds showing FXR agonistic properties have been tested. Among these, Px-102 has shown a dose-dependent reduction in the plasma TGs and cholesterol in *db/db* mice; based on its encouraging results, phase I clinical tests are currently underway[123]. A new molecule activating simultaneously FXR and TGR5 is also being studied. In fact, the BA-derived dual agonist INT-767 in *db/db* mice has been shown to improve hepatic histological features[124]. Moreover, obeticholic acid (OCA), a semi-synthetic bile acid derivative, is being tested in patients with biopsy-proven NASH (ClinicalTrials.gov Identifier: NCT01265498)[125] and in patients with type 2 diabetes mellitus and nonalcoholic fatty liver disease. A phase 2 trial has shown that the administration of 25 or 50 mg OCA for 6 wk was well tolerated, increased insulin sensitivity, and reduced markers of liver inflammation and fibrosis[126]. Thus, several studies supporting these preclinical studies are needed.

Thyroid hormone analogues and derivatives

Thyroid hormones [THs; thyroxin (T4) and 3,3',5-triiodo-L-thyronine (T3)] are potent regulators of the glucose and lipid metabolisms and of body weight and have some potentially therapeutic actions, including decreased weight, plasma cholesterol levels and tissue adiposity. In fact, T3 increases the expression of several genes involved in hepatic lipogenesis and fatty acid oxidation. Thyroid hormones exert their physiological effects by binding to specific nuclear receptors [thyroid hormone receptors (TR)], of which the TRβ isoform is liver-specific and has been considered a putative target for the treatment of dyslipidemia and fatty liver. Recently, it has been discovered that, in the liver, the activation of the NAD^+-dependent deacetylase sirtuin 1 (SIRT1) facilitates fatty acid oxidation in mice lacking SIRT1, all of which continue to develop liver steatosis[127]. Recently, SIRT1 has been reported to interact directly with TRβ1, contributing to the T3-mediated stimulation of hepatic genes[128]. Many T3 actions are tissue-specific, and several agents have been shown to have selective hepatic activities. TRβ selective agonists have been prepared and tested on a variety of experimental models[129]. Two molecules have shown great anti-steatotic and anti-hyperlipidemic effects: GC-1 (or sobetirome) and MB07811. In rats on a CMD diet, it was shown that the co-administration of T3 resulted in a complete regression of liver steatosis associated with a decrease in lipid peroxidation prevents and that the hepatic symptoms could be reversed. GC-1 had greater effect than T3 without generating any significant side effects on heart rate, even while reversing fat accumulation and ameliorating steatohepatitis[130]. Iodiothyronine3,5-diiodo-L-thyronine (T2) is particularly interesting because of its effects on humans of increasing the basal metabolic rate and reducing body weight without generating side effects on the cardiac rate or thyroid axis[131]. The administration of T2 to mice on a High Fat Diet (HFD) is able to prevent and reduce visceral fat accumulation, steatosis, serum TG levels, cholesterol, and the onset of IR without toxic effects on the heart rate and thyroid[132-134]. A novel functional analogue of 3,5-diiodo-L-thyronine TRC150094 has been tested in animal models of overweight and related disorders, including primarily fatty livers. When administered orally to obese Zucker rats, TRC150094 is able to reduce hepatic steatosis[135] by inducing a significant increase in mitochondrial respiration and an increased fatty acids oxidation.

Enzyme modulation

Studies investigating the patho-physiology of liver fat

accumulation have suggested a new therapeutic field to prevent and improve fatty liver: enzymatic modulation. In fact, the modulation of the enzymes that play a role in the oxidation of FFAs can prevent the accumulation of TGs in the liver, counteracting hepatic steatosis, obesity, metabolic disorder and its complications. The enzyme AMPK is well known to regulate mitochondrial long-chain fatty acid oxidation through the inhibition of acetyl-CoAcarboxylase 2 (ACC2)[136]. A product of ACC2 is malonyl-CoA, which is a potent inhibitor of carnitine palmitoyltransferase 1 (CPT1), a mitochondrial membrane enzyme that controls beta-oxidation. Fatty acids are oxidized in a carnitine-dependent manner in cardiac endothelial cells. This evidence suggests that this enzyme plays a role in preventing the inflammation and coagulation associated with metabolic syndrome and heart disease[137]. CPT1 and ACC2 could be studied in the future as potential therapeutic targets to prevent and improve NAFLD, NASH and the metabolic syndrome.

Bullet points

Recently, different drugs obtained by investigating new fields of research have been proposed for the treatment of fatty liver disease. The main fields of research are as follows: (1) inhibition of caspases; (2) agonism and antagonism of the "adenosine system"; (3) PPAR alpha and delta; (4) peripheral cannabinoid 1 receptor agonism; (5) FXR agonism; (6) thyroid hormone analogues and derivatives; and (7) enzyme modulation. Interesting and promising results are being carried out, but several studies to support these data are mandatory.

CONCLUSION

A series of pharmacological agents are currently being investigated for the treatment of patients with NAFLD/NASH. Insulin resistance remains the pivotal alteration responsible for liver disease generation and progression, and the improvement of insulin sensitivity remains an area of intensive research. The administration of therapy in metabolic syndrome and the prevention of its risk factors (*e.g.*, obesity, insulin resistance, hyperlipidemia, hypertension, and inflammation) are being considered as novel targets for NAFLD therapy because of the direct or indirect link of the metabolic syndrome in inducing NAFLD. In fact, metabolic syndrome itself is one of the most important risk factors for NAFLD, and *vice versa*. Regarding this consideration, treatments that effectively improve metabolic syndrome will clearly generate positive outcomes with respect to NAFLD. In fact, similar to NAFLD, lifestyle modification is the first and most effective treatment for metabolic syndrome. Still, given that the etiology and pathogenesis of NAFLD are complex and multifactorial, perhaps we will never find a single therapy for all patients NAFLD. In the future, therapy will be "built on the individual patient"; in other words, we need to "personalize the therapy of NAFLD".

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