The Role of Medications for the Management of Patients with NAFLD

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KEYWORDS

• Nonalcoholic fatty liver disease • Drug treatment • Antioxidants • Insuin-sensitizers
• PPAR agonists • Anti-fibrotic agents

KEY POINTS

- There is a recognized clinical need for an effective treatment of nonalcoholic fatty liver disease (NAFLD); current approaches remain suboptimal and no drug has so far been approved by International Agencies.
- Several factors complicate the development of novel pharmacotherapies, particularly the imprecision of surrogate markers, making histologic assessment compulsory.
- Incretin mimetics, farnesoid x-receptor blockers, peroxisome proliferator activated receptor α/δ agonists, and lysyl oxidase-like-2 inhibitory monoclonal antibodies are currently under scrutiny in randomized controlled trials.
- Although indicated by clinical guidelines, a careful follow-up and treatment of NAFLD is not the rule in the community. If, when, and how long drug therapy should be instituted and continued to reduce the burden of disease are being researched.

INTRODUCTION

Lifestyle changes are a mandatory strategy for the prevention and treatment of nonalcoholic fatty liver disease (NAFLD), but the results depend on individual subjects and therefore are largely unpredictable. Also, subjects who achieve a marked reduction

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of body weight tend to regain weight along the years; in this case recurrence and/or progression of disease may be very likely. This finding stimulated intensive research on pharmacologic treatment strategies and several randomized controlled trials having histology as treatment outcome have been published.[1–11] Several classes of drugs have been tested in the last 10 years, acting at different levels along the sequence of events from pure fatty liver to advanced disease (**Fig. 1**), but no drug has been so far been approved for the treatment of NAFLD. This finding opens a series of challenging questions that may be summarized, such as if, when, and how long should treatment be instituted/continued, considering that also with drugs the results are far from optimal? The situation is similar to that observed in other metabolic disorders largely linked to unhealthy lifestyles, namely, type 2 diabetes and obesity. International guidelines on the treatment of type 2 diabetes have never reached a general consensus as to the need to institute immediate pharmacologic treatment—with well-defined, effective, and safe drugs—soon after diagnosis, unless at risk of acute complications. In obesity all guidelines recommend systematic behavior treatment of weight loss before drug therapy—and very few drugs are approved by International Agencies. Drug therapy may also be effectively superimposed to drugs to increase the final results.[12]

The current scientific evidence on the principal drugs tested so far in several randomized controlled trials, divided according to their prevalent mechanism of action, is presented in **Table 1** and is reviewed in this chapter.

INSULIN SENSITIZERS

As insulin resistance is the basis for liver fat accumulation, insulin sensitizers probably remain the best pharmacologic option for NAFLD treatment.

Metformin

Metformin is a biguanide used widely in clinical practice as a first-line treatment for patients with type 2 diabetes mellitus for over 50 years. Metformin reduces blood

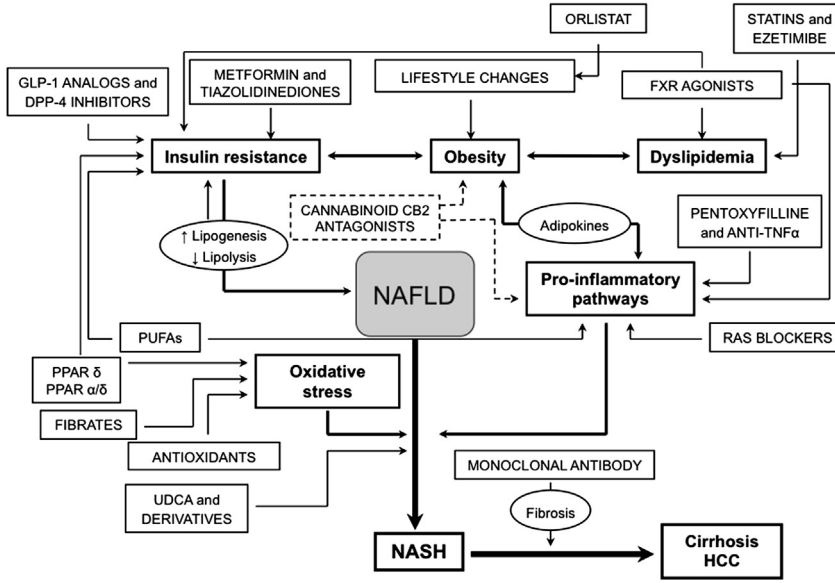

Fig. 1. The complex network of NAFLD pathogenesis and treatment.

Table 1
Principal randomized controlled trials of medication use in subjects with NAFLD

First Author,[Ref.] Year	Treatment	No. of Cases	Duration (mo)	Results (Comparison vs Control)
Lindor et al,[1] 2004	UDCA (13–15 mg/kg/d) vs PL	80/86	24	126 cases completed the 2-y treatment and had a second biopsy. No outcome difference between groups
Bugianesi et al,[2] 2005	MET (2 g) vs Vit. E (800 IU) or prescriptive diet	55/28/27	12	No difference between Vit. E and prescriptive diet, considered control group. ↓ALT and AST with metformin. Second biopsy only in 17 meformin cases: ↓steatosis, ↓necroinflammation and ↓fibrosis
Dufour et al,[3] 2006	UDCA (12–15 mg/kg) + Vit. E (400 IU) or UDCA/PL or PL/PL	15/18/15	24	↓AST and ALT with UDCA + Vit. E; ↓activity score with UDCA + Vit. E (P<.05), mostly as effect of ↓steatosis
Belfort et al,[4] 2006	PIO (45 mg) vs Placebo	26/26 with IGT/DM	6	↓ALT; Improved insulin sensitivity; ↓Steatosis (P = .003) and necroinflammation (P = .001); no difference in fibrosis (P = .08)
Ratziu et al,[5] 2008	ROSI (8 mg) vs Placebo	32/31	12	↓ALT; ↓Steatosis (no other improvement in histology)
Aithal et al,[6] 2008	PIO (30 mg) vs Placebo	37/37	12	↓ALT (P = .009); ↓γ-GT (P = .002); ↓Ferritin; second biopsy in 31/30 cases: ↓hepatocellular injury (P = .005), ↓Fibrosis (P = .05)
Haukeland et al,[7] 2009	MET (mean, 2.6 g) vs PL	24/24	6	No difference in liver biochemistry, insulin resistance, and histology between groups (second biopsy in 44 cases; 20 on metformin)
Leuschner et al,[8] 2010	UDCA (23–28 mg/kg) vs PL	91/94	18	↓ALT; second biopsy in 137 cases: ↓lobular inflammation, no difference in fibrosis (P = .133)
Sanyal et al,[9] 2010	PIO (30 mg) vs Vit. E (800 IU) vs PL	80/84/83	24	↓Steatohepatitis in the Vit. E arm (P = .001), not in the pioglitazone arm (P = .04); ↓lobular inflammation and steatosis with both treatment; no effect on fibrosis (P = .12 and P = .24, respectively)
Ratziu et al,[10] 2011	UDCA (28–35 mg/kg) vs PL	61/55	12	↓ALT; ↓Glycemia; ↓Insulin resistance; ↓Fibrotest (P<.001)
Torres et al,[11] 2011	ROSI (8 mg/d) vs ROSI (4 mg) + MET (500 mg) vs ROSI (4 mg) + Losartan (50 mg)	41/49/45	12	↓ALT in all groups, without differences; 108 cases had a second biopsy (31/37/40). Improvement in steatosis, necroinflammation, ballooning, and fibrosis in all groups ($P \leq .001$), without differences between groups

Abbreviations: DM, diabetes mellitus; IGT, impaired glucose tolerance; MET, metformin; PIO, pioglitazone; PL, placebo; ROSI, rosiglitazone.
Data from Refs.[1–11]

glucose by decreasing hepatic gluconeogenesis, by stimulating glucose uptake in the muscle, and by increasing fatty acid oxidation in adipose tissue. The final effect is an improvement of peripheral insulin sensitivity.

Following a seminal study in 2001,[13] a few clinical trials have reported a beneficial effect of metformin in NAFLD, but limited data are available on histology; metformin led to some improvements in steatosis and necroinflammation, but not in fibrosis. In most studies the changes seen with metformin were not different from those in the control arm and a recent systematic review concluded for a negative effect of metformin on histology.[14] For this reason, the US Guidelines on NAFLD do not support metformin for the treatment of adult NAFLD.[15]

The potential role of metformin has also been examined in pediatric NAFLD patients with results similar to those observed in adults; metformin reduces liver enzymes and improves metabolic parameters, but not histologic features.[16,17]

Metformin treatment also promotes weight loss possibly via appetite control, which makes metformin the first-choice anti-diabetic medication for type 2 diabetes mellitus treatment in obese patients. However, it is unclear whether the benefits of metformin are greater than what might be achieved with weight loss from diet and exercise alone or with a weight loss medication that does not directly affect insulin sensitivity.[18]

The potential beneficial effects of metformin, however, extend outside liver fat. Metformin significantly decreases arterial stiffness, a marker of generalized atherosclerosis, associated with change in circulating adiponectin, a possible marker of the association between liver dysfunction and atherosclerotic vascular disease in patients with NAFLD. Furthermore, metformin has anticancer properties and is being tested to prevent primary cancer in several at-risk conditions. For all these reasons, metformin use might be re-evaluated in NAFLD.

Glitazones (Thiazolidinediones)

Thiazolidinediones (TZDs) have a significant effect on insulin sensitivity in insulin-resistant states and in type 2 diabetes mellitus, as well as in patients with fatty liver or nonalcoholic steatohepatitis (NASH).

TZDs (troglitazone, rosiglitazone, and pioglitazone) are a class of peroxisome proliferator activated receptor γ (PPAR-γ) agonists notable for the ability to cause differentiation of pluripotent stem cells into adipocytes. PPARs are predominantly expressed in adipose tissue, but are also present in muscle, liver, pancreas, heart, and spleen. TZDs treatment increases plasma adiponectin levels and has been shown in patients with type 2 diabetes and those with NASH. Patients with NASH have low plasma adiponectin levels, which are inversely related to insulin resistance and hepatic triglyceride content and are independent of the degree of obesity or glucose tolerance status; the increase in plasma adiponectin levels could mediate some of the insulin-sensitizing effects of PPAR-γ agonists,[19] adding to their anti-inflammatory effects in patients with NASH.

TZDs are probably the best pharmacologic option for subjects with NAFLD. Three large randomized controlled trials reported a beneficial effect of pioglitazone on liver histology, although the advantage was limited for fibrosis.[6,8,9] Rosiglitazone proved effective only on steatosis and liver enzymes, without an effect on necroinflammation and fibrosis.[7] Continuing use of TZDs does not further improve the effects on histology,[20] which are lost after treatment is stopped (**Box 1**).[21]

In conclusion, the efficacy of insulin sensitizers (particularly TZDs), strictly dependent on increased insulin sensitivity, is proven, although limited. Whether they need to be used in association with hepatoprotective agents in individual patients, to maximize the anti-inflammatory and antifibrotic activity, must be defined. There is now solid

Box 1
Insulin sensitizers—mechanism of action

Metformin

- Activation of adenosine monophosphate-activated protein kinase, a regulator of energy metabolism
- Reduced hepatic gluconeogenesis via inhibition of the sterol regulatory element-binding protein-1c (SREBP-1c)
- Adipokine synthesis or secretion

Tiazolidinediones

- Adipocyte differentiation and adipogenesis
- Modification of adipose tissue distribution, with decreased visceral fat, including hepatic fat, and increased peripheral adiposity associated with weight gain
- "Browning" of adipose tissue mitochondria
- Stimulation of fatty acid oxidation and inhibition of hepatic fatty acid synthesis
- Improved insulin signaling and increase in adiponectin levels

evidence for their use,[22] mitigated by undesired side effects (weight gain) and also adverse events.

LIPID-LOWERING DRUGS, ANTIOXIDANT AND HEPATOPROTECTIVE AGENTS

Several studies confirm a link between altered hepatocyte cholesterol metabolism and hepatic-free cholesterol accumulation and NAFLD development and progression. Dietary lipid intake is also an important cofactor in NAFLD development and progression,[23] as in some genetic variants linked with lipid metabolism, like the patatin-like phospholipase domain-containing protein 3,[24] supporting the concept that drugs used for lipid control may be an effective treatment of NAFLD.[25] Reducing lipid levels may also be important to reduce peroxidation, also achieved by different drugs.

The adipose tissue is considered a metabolically active endocrine organ producing pro-inflammatory cytokines, including tumor necrosis factor-α and interleukin-6 and -8, and there is evidence for the activation of other inflammatory pathways and oxidative stress, acting as a "second hit" in the transition between simple fatty liver and steatohepatitis (NASH). Excessive fat accumulation in the liver, whatever its cause, may increase the production of reactive oxygen species, leading to lipid peroxidation and immunologic dysfunction, which prompted testing the effectiveness of antioxidant and cytoprotective compounds, potentially stopping hepatocyte damage (**Box 2**).

Statins

By their activity on hydroxymethylglutaryl CO_2 reductase, statins effectively reduce cholesterol levels in NAFLD in a dose-dependent manner, but their effects are not limited to cholesterol concentrations. Statins reduce the cardiovascular risk, the main cause of death in NAFLD, and control the inflammatory mechanisms involved in NAFLD pathogenesis.[26,27]

The use of statins in NAFLD received additional attention after the publication of the GREACE study, the first randomized controlled trial showing therapeutic benefit on clinical endpoints in NAFLD.[23] In a post-hoc analysis, the use of statins in patients with high transaminase levels presumably due to NAFLD effectively reduced the

Box 2
Lipid-lowering drugs, antioxidant and hepatoprotective agents—mechanism of action

- Decreased lipotoxicity and improved insulin sensitivity (lipid-lowering drugs)
- PPAR-α activity (fibrates)
- Reduced lipid peroxidation and free radicals scavenging activity (antioxidants)
- Anti-inflammatory properties, including the inhibition of pro-inflammatory cytokine production, translating into reduced apoptosis (pentoxyfilline)
- Modulation of inflammation and fibrogenesis and interference with intrahepatic glycolysis and gluconeogenesis (sylibin)

cardiovascular risk. Atorvastatin was the most widely used drug; pharmacokinetic differences translate into different effectiveness in preventing fibrosis of necroinflammation in NAFLD[26] and also the absence of dyslipidemia.[26]

In NAFLD, statins improved liver enzyme levels,[27,28] without any alleged risk of hepatotoxicity.[27–29] Very few data are available on liver histology; in the only small randomized controlled trial with posttreatment histology, 1-year treatment with simvastatin had no significant effect.[23] Pitavastatin did not improve the severity of hepatic steatosis, whereas atorvastatin improved the grade of steatosis, without conflicting results on fibrosis.[24,27]

Ezetimibe

Ezetimibe reduces the absorption of the cholesterol and its target is the Niemann-Pick C1-like 1 protein. This protein is located in the brush border of the intestine and in the liver and is a sterol transporter that is important for the absorption of the cholesterol in the enterocytes and hepatocytes. The excessive amounts of cholesterol are lipotoxic through activation of the liver X receptor. Therefore, the inhibition of the Niemann-Pick C1-like 1 protein does not only lead to a reduced hepatic cholesterol accumulation, but also to decreased lipotoxicity.

Ezetimibe may be used without any restriction in patients with hepatic diseases. In subjects with NAFLD or NASH, ezetimibe reduced liver enzyme levels and the concentration of inflammatory markers[27,30,31]; in a few reports the histologic features of steatosis, ballooning, and the NAFLD activity score also improved.[27,31,32] As to fibrosis, there is good evidence for improvement in animal models, but more data are needed in humans.[31]

Fibrates

Fibrates (fenofibrate, bezafibrate, gemfibrozil) effectively lower serum triglycerides and moderately increase high-density lipoproteins through binding to and activation of PPAR-α.

PPAR-α, member of the PPAR nuclear receptor subfamily, is highly expressed in the hepatocytes, where it controls genes involved in lipid and lipoprotein metabolism, including the uptake and oxidation of free fatty acids, triglyceride hydrolysis, and up-regulation of reverse cholesterol transport, mediated by apolipoprotein A-I and A-II. Furthermore, fibrates improve insulin sensitivity, stimulate fatty acid oxidation, and inhibit vascular inflammation.

Fenofibrate is commonly used in clinical practice to treat hypertriglyceridemia; in NAFLD it increases the expression of enzymes involved in the catabolism of lipid peroxides and reduces hepatic lipid peroxide content.[33,34] Gemfibrozil decreases serum aminotransferase levels in patients with NAFLD, but no data are available on insulin

resistance and liver histology.[35] Bezafibrate, a PPAR pan-agonist, reduces hepatic lipids and the formation of proinflammatory lipoperoxides; along this line it might be particularly effective in NASH.

In conclusion, fibrates might be effective in NAFLD, at least in subjects with fasting hypertriglyceridemia, preventing lipid accumulation in the liver, NASH, and fibrosis.

Polyunsaturated Fatty Acids

Polyunsaturated fatty acids (PUFA) are major constituents of cell membranes and are particularly susceptible to free radical-mediated oxidation. There is some evidence that a low intake of n-3 fatty acids may have a role in NAFLD pathogenesis, highlighting a potential therapeutic target.

When compared with controls, individuals with NAFLD have lower polyunsaturated fat intake. The composition of hepatic long chain fatty acids is characterized by a decrease in the relative levels of n-3 PUFA and an increase in the hepatic n-6/n-3 PUFA ratio,[36,37] associated with defective desaturation activity and dietary imbalance, resulting in hepatic steatosis.[38,39]

In humans, fish oil provides a convenient source of essential n-3 PUFA with few side effects and may directly reduce hepatic lipogenesis and steatosis, improving inflammation and hepatocyte injury. Given the well-recognized problems of adhering to lifestyle interventions and of achieving sustainable weight loss, and considering the side effects of pharmacologic agents, dietary fish oil supplementation represents a practical alternative therapy.[37,40]

In NAFLD, the dietary supplementation with long-chain n-3 PUFAs seems to reduce hepatic steatosis safely.[41–43] A recent meta-analysis reported a statistically significant effect of PUFA supplementation on liver fat in 6/7 studies, a significant improvement of alanine aminotransferase (ALT) levels in 2, while aspartate aminotransferase (AST) was unaffected by PUFA. In 5 studies, steatosis was reduced by n-3 PUFA supplementation in the absence of weight loss. Fibrosis, hepatocyte ballooning, and lobular inflammation were reduced in 85% of the patients.[43,44] Collectively, the data support a role for n-3 long-chain PUFA in NAFLD. The same results might be achieved by a diet rich in n-3 PUFA (fish, nuts, almonds, and other natural products).

Orlistat

Orlistat, a reversible inhibitor of gastric and pancreatic lipase, blocks the absorption of approximately 30% of dietary triglycerides. Orlistat improved AST/ALT, cholesterol, and triglyceride levels and reduced the grade of steatosis, inflammation, and fibrosis in an uncontrolled study. Two small trials in humans investigated the effect of orlistat in NAFLD, with negative results.[45,46] Therefore, orlistat might be an effective treatment of NASH only in the setting of significant weight loss, possibly enhanced by a lifestyle program.[47]

Vitamin E

Vitamin E is the most widely used antioxidant in biomedical research studies, but it is also linked to a greater risk of cardiovascular disease in epidemiologic studies.

Several studies have examined the role of supplemental vitamin E in liver disease. Despite the encouraging in vitro work, results from clinical studies are conflicting. At doses of 400 to 1200 IU daily, the administration of vitamin E reduces serum aminotransferases and alkaline phosphatase, both in monotherapy and as add-on to ursodeoxycholic acid (UDCA), and improves NASH, steatosis, and lobular inflammation, but not fibrosis scores, which are only improved by the association with vitamin C.[9,48–50] The recent US Guidelines recommend vitamin E and conclude that

"Vitamin E administered at daily dose of 800 IU/day improves liver histology in non-diabetic adults with biopsy-proven NASH."[15]

Pentoxifylline

Pentoxifylline (PTX) is a methylxanthine derivative and a nonselective phosphodiesterase inhibitor with well-known hemorheologic activity and anti-inflammatory properties; it acts as a free radical scavenger, inhibits pro-inflammatory cytokine production, namely, tumor necrosis factor-α (TNF-α), and reduces apoptosis.

In patients with NASH, PTX treatment for greater than 1 year versus placebo resulted in a statistically significant normalization or improvement of 30% or more in ALT but not in AST.[51,52] In a systematic review including 6 trials, PTX treatment at a dose of 800 mg to 1600 mg per day for 3 to 6 months improved liver enzymes; histology was only improved after 12 months of follow-up.[53] The positive effects on liver fibrosis might be the consequence of reduced oxidized lipid products. The overall methodological quality of the published studies is however relatively weak and larger studies are needed for additional validation.[54]

Sylibin

Silybin is a potent antioxidant representing about 50% to 70% of the silymarin extract of Silybum marianum (milk thistle). Silybin modulates inflammation and fibrogenesis and interferes with intrahepatic glycolysis and gluconeogenesis. As with other flavolignans, limitations of silybin use include low water solubility, low bioavailability, and poor intestinal absorption, but derivatives with improved solubility may overcome these pharmacologic limitations.

Silybin treatment attenuated liver damage and diabetes in animal models of NASH. The synthetic derivative in use in clinical practice is the silybin phytosome complex (silybin plus phosphatidylcholine), coformulated with vitamin E, with much higher bioavailability.

In animal models silybin administration reduces insulin resistance and liver enzymes, as well as hepatic and myocardial damage, at doses similar to those used in humans. Considering the good tolerability of sylibin and its positive effects, further investigation is warranted.

BILE ACIDS AND DERIVATIVES

Ursodeoxycholic Acid (UDCA)

The rationale for using UDCA (a tertiary bile acid) as a broad hepatoprotective agent is based on a large body of preclinical data[55] and on controlled trials (**Box 3**). The

Box 3
Bile acids and derivatives—mechanism of action

- Hepatoprotective effect (UDCA)
- Anti-inflammatory action, mediated by decreased transcription of tumor necrosis factor-α (UDCA)
- Improved insulin sensitivity in muscle tissue and in the liver
- Down-regulation of lipogenic and apoptotic pathways (Nor-UDCA), favoring increased cholesterol efflux
- Protection against bile-salt-induced cellular toxicity (Tauro-UDCA)
- Anti-inflammatory and lipid-lowering activity (UDCA-LPE)

histologic efficacy remains controversial but there is strong evidence of biochemical effectiveness (on ALT), arguing in favor of a broader hepatoprotective effect of UDCA.

Between 1994 and 2008, 4 studies on UDCA treatment were published on NASH. At doses of 12 to 15 mg/kg/d UDCA monotherapy did not produce any significant effect on liver enzyme levels and histology[1]; the combination of UDCA and vitamin E resulted in significant effects on histology.[3] High-dose UDCA (28–35 mg/kg/d) versus placebo improved liver enzymes, glucose, and insulin levels,[8,11] but the UDCA-treated group lost on average 3% of original body weight, possibly contributing to the favorable results.

Although UDCA monotherapy will not be further tested in NASH, UDCA derivatives have shown promising efficacy stronger than UDCA in preclinical models. In a genetic model of NASH, nor-ursodeoxycholic acid, a C23 homolog of UDCA, improved steatohepatitis by down-regulating lipogenic and apoptotic pathways while increasing hepatic cholesterol efflux. Tauro-ursodeoxycholic acid, a hydrophilic conjugate of UDCA, was able to block apoptosis, thus resulting in improved insulin resistance. Finally, a synthetic bile acid-phospholipid conjugate ursodeoxycholyl-lysophosphatidylethanolamide (UDCA-LPE) was designed to target phosphatidylcholine to hepatocytes by means of the bile acid transport systems. In in vivo models of NASH, it reduced hepatic fat overload and inhibited de novo lipogenesis, also reducing proinflammatory pathways and liver enzyme levels.

A recent study confirmed that UDCA-LPE ameliorates hepatic injury in different stages of NAFLD, such as steatosis and advanced steatohepatitis. For the excellent anti-inflammatory and lipid-lowering properties, and inhibition of disease progression, UDCA-LPE represents a promising compound suitable for the treatment of NAFLD.[56]

NEW AREAS OF RESEARCH

Several new areas of research are being exploited or old areas are receiving new interest and developments, to provide more effective and safer drugs for NAFLD treatment (**Box 4**).

Box 4
New areas of research—mechanism of action of new drugs

- Stimulation of the farnesoid X receptor-α that regulates glucose and lipid metabolism (OCA)
- Immunomodulatory and anti-inflammatory action, mediated by the inhibition of nuclear factor-kB and down-regulation of inducible nitric oxide synthase (OCA)
- Increased hepatic insulin signaling and sensitivity (GLP-1 agonists)
- Decreased hepatic lipogenesis and liver triglyceride content (GLP-1 agonists)
- GLP-1 agonist- and DPP-4 inhibitor-mediated protection of pancreatic β-cells from endoplasmic reticulum stress and apoptosis
- Insulin-sensitizing activity in the liver (PPAR-δ agonists)
- Reduced food intake (Endocannabinoid CB2 agonists)
- Improved insulin sensitivity and block of the hepatic recruitment of inflammatory cells and the development of fibrosis (ARB)
- Direct inhibition or even reversal of hepatic fibrosis (Lysyl oxidase-like-2 inhibitory monoclonal antibody)

Obeticholic Acid and Farnesoid X Receptor Agonists

Bile acids are critical regulators of hepatic lipid and glucose metabolism through 2 major receptor pathways: farnesoid X receptor (FXR), a member of the nuclear hormone receptor superfamily, and G protein-coupled bile acid receptor 1 (GPBAR1). FXRs are mainly found in the liver, kidney, and intestines, and overall inhibit hepatic bile acid production.

FXR knockout mice have high plasma triglyceride and cholesterol levels as well as a hepatic phenotype similar to NASH patients,[57] including the possible development of hepatocellular carcinoma (HCC).[58] Signaling through FXR and GPBAR1 modulates metabolic pathways, regulating not only bile acid synthesis and their enterohepatic recirculation but also triglyceride, cholesterol and glucose levels, energy homeostasis, and immune responses.

Obeticholic acid (OCA, INT-747, 6α-ethyl-chenodeoxycholic acid), a semisynthetic derivative of chenodeoxycholic acid, is a natural agonist of the FXR-α nuclear hormone receptor that regulates glucose and lipid metabolism. In animal models, OCA decreases insulin resistance and hepatic steatosis and displays immunomodulatory and anti-inflammatory properties.[59] In a phase 2 trial, OCA administration for 6 weeks was well tolerated, increased insulin sensitivity, and reduced liver enzymes and the markers of liver inflammation and fibrosis in patients with type 2 diabetes and NAFLD.[60] A large US multicenter, 18-month phase IIb study of OCA in NASH patients is currently ongoing. Overall, adverse events were not different in patients on treatment or on placebo.

Incretin Mimetics

The rationale for the use of the glucagon-like peptide-1 analogues (GLP-1a) and the dipeptidyl peptidase-4 inhibitors (DPP-4i) in NAFLD does not only derive from their insulin-sensitizing activity but also from the evidence of a reduced activity of the incretin system in NASH patients. The expression of GLP-1 receptors in liver or hepatocytes is inconsistent in different laboratories, but the expression in the biopsies from NASH patients is generally lower compared with control biopsies,[61] and DPP-4 activity is 30% increased.[62] Notably, both the serum activity and the intensity of DPP-4 immunostaining in the liver are associated with the intensity of fatty infiltration and histologic grading of NASH, providing a rationale for the use of DPP-4i to slow the progression of hepatic steatosis and inflammation.[63] GLP-1a and DPP-4i are also likely to improve NAFLD through improved insulin sensitivity.[64]

The protective effects of incretin-mimetic agents on hepatic steatosis were found in diet-induced obese mice treated with GLP-1 analogues and with DPP-IV inhibitors (in linagliptin-treated diet-induced obese mice liver fat content was reduced by up to 30%),[64–68] but data were not confirmed in patients treated with exenatide.[69] More research is needed to explore the mechanism and the possibility of using incretin-mimetic agents as therapy for NAFLD.[70] Notably, clinical studies have provided evidence that DPP-4i can be used safely without any risk of hypoglycemia even in nondiabetic patients.[63]

PPAR-δ Agonists

The function of PPAR-δ has long been unrecognized. Now PPAR-δ seems to be the most promising of all PPAR targets for its specific action on the liver, muscle, and fat. The liver was only recently identified as a major PPAR-δ-responsive tissue, able to burn large amounts of glucose, thus reducing hyperglycemia and improving insulin sensitivity. PPAR-δ also regulates the catabolism and/or the β-oxidation of lipids in

adipose tissue and muscle, increases the production of mono-unsaturated fatty acids, and may protect the liver from free fatty acid-mediated lipotoxicity and inflammatory response.[71,72]

The lipogenic activity of PPAR-δ has also been observed in human studies.[73] Ligands for PPAR-δ have been proposed to act as insulin sensitizers, based on improvements in standard glucose-tolerance tests. Studies based on long-term ligand treatment regimens show a significant weight loss and a decreased fat mass, conditions potentially responsible for increased insulin sensitivity. Along this line, the PPAR-δ agonist GW0742 was reported to reduce hepatic steatosis and hyperglycemia.[72] In mice fed the steatogenic metionine-choline-deficient diet, the PPAR-δ agonist GW501516 improved hepatic steatosis and reduced inflammation.[74,75] Thus, PPAR-δ might be helpful in NASH,[76] but no selective PPAR-δ agonists are clinically available at present.

PPAR-α/δ Agonists

GFT505 and its main active metabolites are PPAR modulators with preferential activity on human PPAR-α in vitro (half-maximal effective concentration) and with additional activity on human PPAR-δ. After oral administration, it accumulates predominantly in the liver, with concomitant repression of pro-inflammatory and profibrotic genes. Preclinical and clinical data demonstrated that GFT505 treatment improves several metabolic parameters, including fasting plasma glucose and insulin sensitivity (homeostasis model of assessment-insulin resistance) in abdominally obese patients.[77] This improvement in metabolic parameters supports its use in the treatment of hepatic steatosis and the results seem promising. GFT505 treatment decreased plasma concentrations of liver enzymes and had a protective effect on steatosis, inflammation, and fibrosis.[78,79] A randomized, double-blind, placebo-controlled, 1-year phase IIb study (ClinicalTrial.gov identifier NCT01694849) is currently ongoing and will assess the efficacy and safety of GFT505 in patients with histologically proven NASH. No serious adverse events have so far been reported.

Endocannabinoids (Cannabinoid Receptor Blockers Type 1 and Type 2 (CB1 and CB2))

The endocannabinoid system, involved in the regulation of food intake and body weight, represents a target for NASH therapy.[80] Rimonabant was the first selective CB1 receptor blocker introduced into clinical practice. CB1 antagonism also improved obesity-associated dyslipidemia and insulin resistance to a greater extent than expected from weight loss. For this reason, different studies were planned in NAFLD, supported by studies in experimental animals. Unfortunately, the alarming incidence of central side effects, including severe depression,[81] led to rimonabant withdrawal. Contrary to CB1, highly expressed in the brain, CB2 receptors are mainly expressed in the periphery, predominantly by immune cells, and play a key role in inflammatory processes possibly involved in the pathogenesis of obesity-associated insulin resistance and the progression of fatty liver to NASH.[81] Modulation of CB2 receptors is thus emerging as a potential therapeutic strategy, and the development of peripherally acting CB1/CB2 antagonists remains an area of intense research.[82]

Drugs Modulating the Renin-Angiotensin System (RAS)

In the liver, chronic injury up-regulates the local tissue renin-angiotensin system, which contributes to the recruitment of inflammatory cells and the development of fibrosis. Angiotensin receptor blockers (ARBs) might reduce oxidative stress, attenuating the progression of hepatic fibrosis. In human studies, 2 ARBs (losartan and valsartan) reduced transaminase levels[11,83]; one reduced the grade of liver steatosis,

fibrosis, and ballooning, but ARB use never did reach the clinical stage. Nonetheless, they are widely used, with a well-characterized safety profile, in the presence of comorbidities.

Lysyl Oxidase-Like-2 Inhibitory Monoclonal Antibody

Fibroblasts constitute the major cell type of the stromal compartment and contribute to tumor growth, angiogenesis, and fibrotic disease through paracrine signaling. The matrix enzyme lysyl oxidase-like-2 has an important role in the creation and maintenance of the pathologic microenvironment in cancer and fibrotic diseases. The inhibition of this enzyme by a lysyl oxidase-like-2 inhibitor monoclonal antibody (simtuzumab, GS-6624; Gilead Sciences, Foster City, CA, USA) is associated with reduced tumor volume in a mice model, probably due to a reduction of cross-linked collagenous matrix and activated fibroblasts. The use of this monoclonal antibody is also associated with the inhibition of transfer growth factor-β signaling in fibroblasts and reduced porto-portal and porto-central fibrosis. This evidence is the basis for the development of a new class of drugs to be tested in several hepatic diseases characterized by advanced fibrosis/cirrhosis, to reduce directly the progression to fibrotic stage and/or to reverse stable fibrosis.[84] At least 2 phase IIb trials are at present recruiting participants for studies in advanced NASH with/without cirrhosis by the use of GS-6624, infused every 2 weeks for 96 weeks. Outcome results are expected by August 2015.

SUMMARY

There is a definite clinical need for an effective treatment of NAFLD, but current approaches remain suboptimal. Several factors will complicate the development of novel pharmacotherapies, including: (1) the multifactorial pathogenesis of NAFLD, (2) the heterogeneity of the patient population, (3) the imprecision of current disease staging techniques, (4) ill-validated surrogate markers, making histologic assessment compulsory, (5) the slowly progressive nature of NASH and the tendency of a proportion of cases to show spontaneous disease regression, likely related to the improvement of metabolic control.[85]

At present, no drugs have been approved with specific indications for NAFLD; there is however general consensus that continuing clinical research is needed on hard end points (ie, improvement or resolution of NASH), with no worsening of fibrosis and/or improvement of steatosis (quantitatively assessed) and sustained normalization of liver enzymes.[86] Although indicated by clinical guidelines, a careful follow-up and treatment of NAFLD are not the rule in the community. Four questions remain unanswered: (1) Should drug therapy be initiated independently of lifestyle changes? (2) Which drug, if any, in individual patients, according to age, comorbidities, and disease severity? Which drug for NAFLD patients with diabetes, where most putative drugs are already in use, and in normal-weight NAFLD? (3) Should treatment be continued lifelong, in the absence of significant lifestyle changes?

Efforts should be made to close the gap and reduce the future burden of NAFLD and its complications.[87]

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