Pharmacologic Management of Nonalcoholic Steatohepatitis

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Abstract: Nonalcoholic steatohepatitis (NASH) is increasingly recognized as a major form of chronic liver disease in adults and children. Although improved dietary habits and regular exercise remain the primary recommendations for patients with or at risk of having NASH, implementing and sustaining these lifestyle changes have proven to be challenging. Pharmacologic approaches are now being evaluated to prevent the development of cirrhosis and its complications in the approximately 1% of the population of countries consuming a Western diet at risk for NASH. Although some therapies are available for the treatment of NASH, none is currently approved by the US Food and Drug Administration. Approval of new drugs for NASH is expected within the next several years. Thus, a rational approach to understanding how these drugs work is needed. This article explains how the many new therapies that are currently in clinical trials address the varied mechanisms by which patients develop NASH and NASH-induced cirrhosis.

Nonalcoholic steatohepatitis (NASH) is a major form of chronic liver disease and is becoming the leading indication for liver transplantation.[1] In addition to being a major contributor to death from liver disease, NASH imposes a substantial economic burden on health care systems in developed countries.[2] The primary treatment recommendations for patients at risk for NASH or with a documented diagnosis of NASH are to adopt healthy eating habits and to engage in regular exercise.[3,4] However, for a variety of genetic, social, and physical reasons, these recommendations have proven to be difficult to implement and even more challenging for the majority of patients to maintain.[5] Thus, pharmacologic therapies are being evaluated, with the goal of reducing death or the need for liver transplantation for cirrhosis caused by NASH.

There are currently no federally approved drugs for the treatment or prevention of NASH. Drug development for this disease has been challenging because cirrhosis, hepatocellular carcinoma, death, and the need for liver transplantation typically occur decades after disease onset. Regulatory approval requires proof that

treatments assist in reducing these endpoints or show a benefit in reliable surrogate markers that predict the future development of these endpoints.[6] Considerable effort has been directed to the development of such surrogate markers to use in clinical trials.

The development of drugs that may halt or reverse NASH and its associated fibrosis has accelerated over the past 5 years, and the first drug approvals could potentially occur within the next 2 years, followed by the rapid approval of additional options over the subsequent 2 years. The advancement in the treatment of NASH will require clinicians to understand the underlying pathogenesis of the disease and its related fibrosis, as well as how the various drugs are meant to intervene to prevent progression to cirrhosis. This article reviews drugs that have been evaluated in prior clinical trials for NASH or are currently undergoing evaluation in phase 2 or 3 trials. Focus is paid only on the primary mechanisms of action of specific drugs, as the many other potential targets of these agents are beyond the scope of this article. The Figure illustrates how the drugs fit into the various pathways that are responsible for the development of NASH, and it intentionally does not capture the full complexity of the disease or the various potential targets of the treatments shown. Of note, NASH is likely a heterogeneous disease, with patients arriving at a common phenotype by different mechanisms. In the future, there may be an era of personalized medicine during which clinicians can select specific therapies based on a patient's underlying genetic, epigenetic, microbiomic, and environmental risks. Research is needed to better understand how to treat these risks individually.

Currently Available Treatments

No treatment is currently approved by the US Food and Drug Administration to treat NASH. Two of the most-studied therapies in this area are pioglitazone and vitamin E.

Pioglitazone

Patients with NASH often have clinical and laboratory evidence of insulin resistance; therefore, insulin-sensitizing agents have been evaluated in clinical trials for the treatment of NASH. Pioglitazone is a peroxisome proliferator-activated receptor (PPAR) γ ligand, and it also targets the mitochondrial pyruvate carrier proteins or mitochondrial target of thiazolidinediones and possibly PPAR α. Several clinical trials with histologic endpoints have demonstrated that pioglitazone can improve NASH and its associated liver fibrosis in some patients.[7,8] Based on these data, the American Association for the Study of Liver Diseases and the European Association for the Study of the Liver guidelines on NASH state that pioglitazone can be considered in patients with biopsy-proven NASH, taking into account the risks of weight gain, loss of bone density, and bladder cancer.[3,4] The insulin-sensitizing effect of pioglitazone appears to be at the level of adipose tissue, and it reduces inappropriate lipolysis as a source of fatty acids delivered to the liver.[9] By comparison, metformin improves insulin sensitivity in the liver but has not shown benefit as a treatment for NASH in several large clinical trials.[10,11]

Vitamin E

NASH is associated with increased oxidant stress in the liver, but whether oxidant stress is part of the pathogenesis of NASH in humans remains uncertain. One way to prove this hypothesis is to demonstrate that antioxidant agents are effective for treating NASH. Two placebo-controlled studies, one in children[10] and one in adults,[7] did show a benefit of vitamin E, although only in some patients in both groups. However, using therapeutic doses of vitamin E may not be completely benign, as studies have suggested an increased risk of cardiovascular disease.[12,13] The society guidelines recommend that vitamin E be considered in noncirrhotic, nondiabetic patients with biopsy-proven NASH at a dose of 800 IU of the natural form (ie, rrr-α-tocopherol).[3,4]

Pharmacologic Agents in Clinical Trials

Multiple agents that address diverse potential targets are being evaluated in clinical trials (Figure). The target diversity underscores the complexity of the pathogenesis of NASH as well as the likelihood that patients develop the histologic phenotype of NASH through different mechanisms, thus requiring different therapies.

Modulators of Energy Metabolism

A major driver of the pathogenesis of NASH is the oversupply of metabolic energy substrates, primarily carbohydrates and fatty acids, to the liver. Processes that limit energy intake or divert energy substrates to other tissues for storage or catabolism can protect the liver. Behavioral changes (eg, stopping consumption of sugar-sweetened beverages), satiety-modulating agents, and bariatric surgery are methods to decrease energy intake. Exercise also promotes the disposal of energy substrates in muscle and effectively diverts them from the liver to prevent or reverse NASH.[14] Drugs that promote energy disposal through mitochondrial uncoupling with thermogenesis in tissues such as brown adipose tissue may also prove beneficial.[15] Endogenous mechanisms control the regulation of energy efficiency, and their pharmacologic manipulation is being sought as an
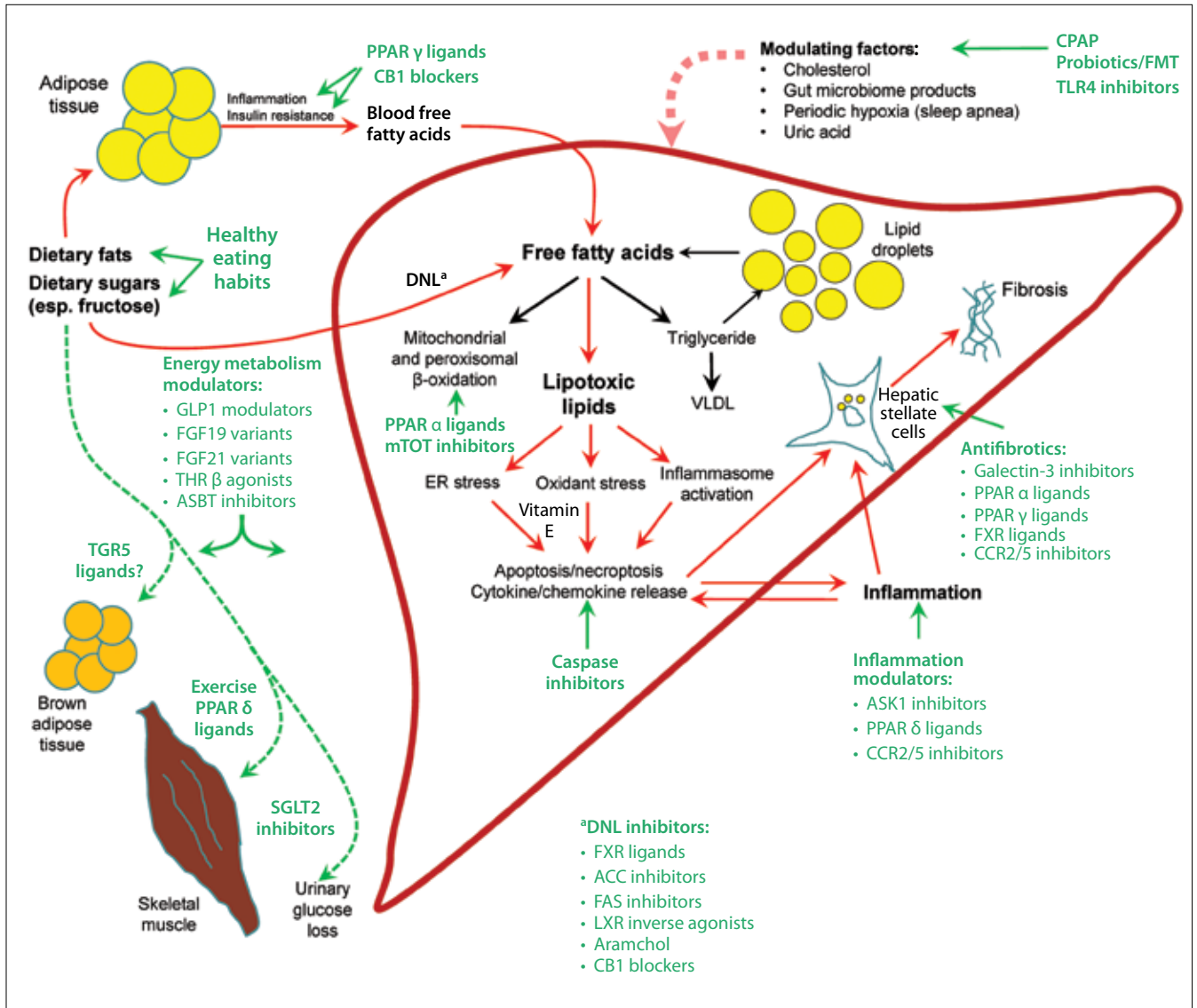
Figure. The substrate overload lipotoxic liver injury model illustrates the pathogenesis of nonalcoholic steatohepatitis (NASH) and targets of therapy. Based on extensive cell culture, animal studies, and human trials, free fatty acids play a central role in the pathogenesis of NASH. Free fatty acids can come from lipolysis of triglyceride in adipose tissue, and are delivered to the liver through the blood. A significant contributor to the free fatty acid flow through the liver is the process of de novo lipogenesis (DNL), whereby hepatocytes synthesize new fatty acids from excess carbohydrates, especially fructose. Fatty acids in hepatocytes can be metabolized by mitochondrial and peroxisomal β-oxidation and converted into triglycerides. Triglycerides can then either be excreted into the blood as very-low-density lipoprotein (VLDL) or stored in lipid droplets. Lipid droplet triglyceride undergoes regulated lipolysis to release fatty acids back into the hepatocyte–free fatty acid pool. When the disposal of fatty acids through β-oxidation or the formation of triglyceride is impaired or overwhelmed by substrate overload, fatty acids can be converted into a number of lipotoxic species that lead to endoplasmic reticulum (ER) stress, oxidant stress, and inflammasome activation. These processes cause the cell injury, inflammation, stellate cell activation, and progressive accumulation of excess extracellular matrix that characterize NASH. Lifestyle modifications focused on healthy eating habits and regular exercise reduce the substrate overload by decreasing intake and diverting energy substrates to metabolically active tissues such as skeletal muscle, thus preventing or reversing NASH. A number of pharmacotherapies are currently being evaluated in clinical trials and are shown with their primary targets. DNL is a target of many therapies by downregulation of the enzymes of DNL (eg, ACC inhibition) or reduction of the expressions of the enzymes of DNL (eg, FXR ligands).

ACC, acetyl coenzyme A carboxylase; ASBT, apical sodium-dependent bile acid transporter; ASK1, apoptosis signal-regulating kinase 1; CB1, cannabinoid receptor 1; CCR, C-C motif chemokine receptor; CPAP, continuous positive airway pressure; FAS, fatty acid synthetase; FGF, fibroblast growth factor; FMT, fecal microbiota transplant; FXR, farnesoid X receptor; GLP1, glucagon-like peptide-1; LXR, liver X receptor; mTOT, mitochondrial target of thiazolidinediones; PPAR, peroxisome proliferator-activated receptor; SGLT2, sodium-glucose cotransporter 2; TGR5, Takeda G-protein coupled receptor-5; THR, thyroid hormone receptor; TLR4, toll-like receptor 4.

indirect way to control energy efficiency. If such agents are found to be safe and effective, they would not only benefit the liver but also reduce obesity and likely treat other components of metabolic syndrome.

Incretin Axis–Modulating Agents Glucagon-like peptide-1 (GLP1) is a gut hormone that is released by enteroendocrine cells in response to nutrients in the small bowel after a meal. Its canonical function is to stimulate insulin release by the pancreas, but research has shown diverse metabolic effects that likely underlie many of its benefits in patients with type 2 diabetes mellitus.[16]

The GLP1 receptor agonist exenatide was found to lower alanine aminotransferase (ALT) levels in a diabetes trial,[17] and other incretin axis–modulating agents have been evaluated as therapies for NASH. A trial of the GLP1 receptor agonist liraglutide (Victoza, Novo Nordisk) resulted in the resolution of NASH in 39% of patients compared to 9% treated with placebo[18]; however, the trial was relatively small (45 patients with end-of-treatment biopsies), and these results need to be validated in larger studies. A study of a more potent GLP1 receptor agonist, semaglutide (Ozempic, Novo Nordisk), is underway. Endogenous GLP1 is catabolized by dipeptidyl peptidase-4, and inhibitors of this enzyme (gliptins) are also currently in trials for NASH. Early data have been equivocal on their benefit in NASH.

Fibroblast Growth Factor 19 Fibroblast growth factor (FGF) 19 is a peptide hormone released by enterocytes in response to activation of the farnesoid X receptor (FXR) by bile acids. Through FGF19 receptors in the liver, the hormone downregulates lipogenesis; through receptors in adipose tissue and the central nervous system, it increases fat oxidation, improves insulin sensitivity, and indirectly reduces hepatic lipogenesis. Native FGF19 has mitogenic properties, raising concerns that pharmacologic supplementation with supraphysiologic levels may support carcinogenesis. In a preliminary trial, a nonmitogenic FGF19 variant was shown to reduce liver fat, serum ALT levels, and serum fibrosis markers in NASH.[19]

Fibroblast Growth Factor 21 FGF21 is another peptide hormone, and is produced by the liver with beneficial metabolic effects in insulin sensitivity and food intake. A study of a long-acting pegylated FGF21 compound in patients with NASH demonstrated decreased liver fat content, improved serum ALT levels, and improved serum fibrosis markers.[19] Based on these data, further studies are being undertaken to assess the effect of the FGF21 analogue on liver histology in patients with NASH.

Thyroid Hormone Receptor β Activation Thyroid hormone regulates diverse metabolic processes throughout the body with increased oxidative disposal of metabolic substrates; however, augmenting thyroid hormone responses with the native hormone can lead to adverse effects attributable to the activation of thyroid hormone receptor (THR) α. In animal models, activation of the liver-specific THR β increases oxidative metabolism in the liver without the systemic side effects, and a human study demonstrated beneficial effects on serum lipids.[20] A trial of a small molecule activator of this receptor in patients with NASH has been undertaken, and the preliminary results are promising.[21]

Manipulating Bile Acid Homeostasis Bile acids are essentially detergent molecules made by the liver and secreted into bile to facilitate intestinal fat digestion. However, low levels of bile acids are also found in the circulation, where they exert important metabolic effects mediated by the Takeda G-protein coupled receptor-5 (TGR5).[22] TGR5 is found in brown adipose tissue and promotes uncoupled oxidative pathways that generate heat instead of adenosine triphosphate (ATP) and, thus, facilitates the disposal of metabolic substrates with associated thermogenesis. TGR5 is also the receptor on enteroendocrine cells that promotes the release of GLP1 from enteroendocrine cells, thus linking bile acid homeostasis with the incretin pathway described previously. The serum bile acid profile in NASH patients is not normal,[23] and animal research of drugs that impair the normal uptake of bile acids in the terminal ileum suggest beneficial metabolic effects of reducing the bile acid pool.[24] The actual mechanisms of these effects remain largely unknown, but clinical trials are currently examining the effects of disrupting the normal enterohepatic recirculation of bile acids using inhibitors of the apical sodium-dependent bile acid transporter inhibitor or impairing bile acid synthesis with FXR ligands in patients with NASH.

Cannabinoid Receptor 1 Blockade Endogenous and exogenous agonists of cannabinoid receptor 1 (CB1) are known for their euphoric effects mediated by receptors in the central nervous system. CB1 is found throughout the body, including in the liver, where it mediates substantial effects on metabolism.[25] CB1 antagonists have beneficial effects on metabolism in animal studies, which have been confirmed in preliminary human studies.[24,26] However, a trial of a CB1 antagonist in patients with NASH was halted due to dysphoric effects, and newer agents that cannot cross the blood-brain barrier are now in the initial stages of evaluation.

Increase of Metabolic Substrate Disposal Sparing the liver from receiving fatty acids or carbohydrates or diverting fatty acids to oxidative pathways within the

liver are potential therapeutic avenues to prevent energy substrates from promoting the generation of toxic lipid species in the liver that lead to NASH. Exercise is one way to dispose of energy substrates, but other pathways are also viable targets for drug therapy.

Brown Adipose Tissue Activation Brown adipose tissue is a highly metabolically active type of adipose tissue found in focal areas throughout the body.[27] Although its role in metabolism and whether it is present in all humans continues to be debated, the presence and activation of brown adipose tissue is associated with a decreased risk of metabolic disease. As described previously, bile acids activate substrate utilization and thermogenesis by brown adipose tissue, and studies are underway to identify other activators of this tissue. Interestingly, serotonin interferes with the activation of brown adipose tissue, raising the question of whether blocking serotonin synthesis would have beneficial metabolic effects.[28]

Peroxisome Proliferator-Activated Receptor δ PPAR δ is a nuclear receptor found in muscle (where it increases fatty acid oxidation), in macrophages (where it mediates polarization away from the proinflammatory M1 phenotype to the anti-inflammatory M2 phenotype), and in stellate cells (where it may modulate fibrogenesis).[29] PPAR δ ligands are being evaluated as therapeutic agents in a number of disorders; one agent is currently in trials for NASH. Some drugs, such as elafibranor (GFT505, Genfit), have both PPAR α and δ activity, and elafibranor has shown promise in an early study.[30] Drugs with pan-PPAR activity are also being evaluated.

Sodium-Glucose Cotransporter Inhibitors A conceptually simple way to divert energy substrates away from the liver is to promote urinary glucose loss by inhibiting reuptake of glucose from the glomerular filtrate in the nephron. This reuptake is mediated by sodium-glucose cotransporter (SGLT) 2. Inhibitors of SGLT2 have become commonly used as an adjunct in the treatment of type 2 diabetes. It has been estimated that patients treated with SGLT2 inhibitors lose approximately 240 to 320 kCal of glucose in the urine daily; thus, the net metabolic benefit is not trivial.[31] SGLT2 inhibitors are being evaluated for the treatment of NASH, as they may play an adjunctive role in this area as well.

Inhibition of De Novo Lipogenesis
Most of the fatty acid flux through the liver originates from fatty acids that have been released by adipose tissue lipolysis followed by their delivery to other tissues, such as the liver, where they are used as an energy source. However, a fraction of the fatty acids that the liver must handle is synthesized in hepatocytes through the process of de novo lipogenesis (DNL). In NASH patients, DNL can contribute up to 25% of the fatty acid flux through the liver.[32] The primary substrates for DNL are carbohydrates. Glucose can feed into DNL, but the liver also regulates how much glucose it takes up, and, if the liver takes up excess glucose, it can also store glucose as the polymer glycogen. By comparison, fructose is almost completely taken up by the liver on first pass from the portal circulation, and it is committed to the pathway of DNL without regulation or other disposal routes. These points emphasize the need for NASH patients to avoid consumption of high-sugar foods (sucrose is half fructose) or beverages containing sucrose or high-fructose corn syrup (which is typically 55% fructose). Even with these dietary interventions, endogenous glucose still contributes substantially to DNL. Therefore, pharmacologic inhibition of DNL has been pursued as a means to treat NASH.

Farnesoid X Receptor Ligands The primary function of the bile acid–sensing nuclear FXR is to sense the presence of excess bile acids and decrease their uptake, inhibit their synthesis, and increase their secretion from hepatocytes into the bile. FXR signaling increases the expression of the inhibitory nuclear receptor small heterodimer protein (SHP) 1, which effectively downregulates the nuclear receptor sterol regulatory element-binding protein (SREBP) 1c. SREBP1c is the master regulator of the expression of genes responsible for DNL: acetyl coenzyme A carboxylase (ACC), fatty acid synthetase, and stearoyl coenzyme A desaturases. Treatment with FXR ligands increases SHP1, which decreases SREBP1c and leads to decreased fatty acid synthesis in the liver. Multiple FXR ligands are in various stages of evaluation, and obeticholic acid (Ocaliva, Intercept Pharmaceuticals) is now in a large phase 3 trial based on promising results in a phase 2b study.[7]

Acetyl Coenzyme A Carboxylase Inhibitors ACC is the first step in converting acetyl coenzyme A derived from carbohydrate metabolism into fatty acids. A liver-specific inhibitor of ACC1 was shown to significantly decrease DNL in humans,[33,34] although it was also shown to increase serum triglycerides in a phase 2 trial.[35] This inhibitor is now in combination trials with selonsertib (GS-4997, Gilead Sciences). The asymptomatic increase in serum triglycerides that is seen in some patients has subsequently been investigated and has been attributed to a combination of decreased lipoprotein lipase activity and an increase in very-low-density lipoprotein secretion or delayed chylomicron clearance. In the phase 2 trial, treatment with a fibrate or fish oil was shown to lower

the triglyceride levels in patients who had an elevated amount.[35] One benefit of inhibiting DNL at the level of ACC is that it reduces the production of malonyl coenzyme A, thus allowing unimpaired disposal of fatty acids through mitochondrial β-oxidation.

Stearoyl Coenzyme A Desaturase Inhibitors Aramchol (Galmed Pharmaceuticals) is a bile acid–fatty acid conjugate that was developed to dissolve gallstones but in preliminary animal studies was found to improve fatty liver. Further evaluation demonstrated that the agent inhibits the stearoyl coenzyme A desaturases, the phase in DNL that introduces double bonds into fatty acids, thereby inhibiting DNL. A phase 2 clinical trial demonstrated that the agent improved liver fat but did not lower serum ALT levels.[36] This agent is now being evaluated in a phase 2b clinical trial with histologic endpoints.

Liver X Receptor Inverse Agonists The liver X receptor (LXR) is a nuclear receptor that has generally the opposite effects of FXR.[37,38] LXR senses cholesterol levels via oxysterol levels; increases DNL by increasing SREBP1c expression, bile acid synthesis, and energy disposal; and modulates inflammation. Under development are inverse agonists for LXR that act as inhibitors of LXR-mediated gene expression to subbasal levels, thus inhibiting the stimulatory effects of endogenous ligands. One such LXR inverse agonist is currently in a phase 2 clinical trial for NASH, and how it fares may depend on the many diverse effects for LXR inhibition.

Augmentation of Mitochondrial Function
Mitochondria use metabolic substrates to generate ATP; if the electron transport chain is uncoupled from generating ATP, mitochondria generate heat (thermogenesis). Augmenting energy disposal through mitochondrial metabolism is a logical target for pharmacotherapy. Choosing this route assumes that the mitochondria are healthy and can handle the burden. There are some data to suggest that mitochondria in certain patients with NASH are dysfunctional.[39,40] The nuclear receptor PPAR α regulates mitochondrial biogenesis, and PPAR α activators such as fibrates are often used to treat dyslipidemia. Whether these agents have an adjunctive role in the treatment of NASH is now being explored with a number of drugs that target PPAR α, among other PPARs. For example, elafibranor, a PPAR α and δ ligand, was found to improve NASH in patients with more advanced disease in a phase 2 study[30] and is now being evaluated in a large phase 3 trial. Targeting the mitochondrial pyruvate carrier protein is also being evaluated, as it was discovered that inhibition of this

carrier may contribute to the benefits of pioglitazone in NASH.[41]

Anti-Inflammatory and Antiapoptotic Approaches
A histologic hallmark of NASH is the presence of lobular inflammation. Studies have shown that hepatocyte apoptosis is a major mechanism of cell death in NASH.[42] Both processes provide the necessary stimuli for the activation of hepatic stellate cells to produce excess collagen, which leads to progressive fibrosis, cirrhosis, and death or the need for liver transplantation. Thus, therapeutic approaches to decrease inflammation and apoptosis, or the consequences of these processes, are being evaluated as treatments for NASH.

Pancaspase Inhibitor Inhibiting apoptosis with the pancaspase inhibitor emricasan (IDN-6556 or PF-03491390, Conatus Pharmaceuticals) was found to improve liver enzymes in patients with hepatitis C virus infection.[43] Because apoptosis is an important part of NASH, multiple studies of emricasan are currently underway in patients with the spectrum of NASH, including decompensated cirrhosis.

Apoptosis Signal-Regulating Kinase 1 Inhibitor Apoptosis signal-regulating kinase 1 (ASK1) plays a central role in sensing oxidant stress and activating stress pathways that are mediated through the phosphorylation of key signaling kinases p38 and c-Jun N-terminal kinase. Selonsertib, an orally available inhibitor of ASK1, was found in a phase 2 study to reduce liver fibrosis at a relatively early 6-month time point.[44] This drug is now being evaluated as a single agent in two phase 3 studies, one in patients with stage 3 fibrosis and the other in patients with established but well-compensated cirrhosis. Studies have also been initiated using selonsertib in combination with a FXR ligand and an ACC inhibitor to determine if there are synergistic benefits of treating multiple targets with these drugs.

Chemokine Receptor 2 and 5 Inhibitors Inhibitors of the C-C motif chemokine receptors 2 and 5 (CCR2/5) were developed as potential treatments for HIV infection, and studies in this patient population demonstrated potential benefits on liver markers. CCR2 and 5 mediate key aspects of the inflammatory signaling in the liver, and receptor inhibition revealed benefits in mouse models of NASH. A clinical trial of the CCR2/5 inhibitor cenicriviroc (Allergan) demonstrated no significant improvement in NASH, but it did appear to improve fibrosis in some patients.[45] Based on these results, the drug is now being further evaluated in a large phase 3 trial.

Antifibrotics

Ideally, therapies will be developed that address the upstream metabolic abnormalities that lead to NASH and the liver-specific processes that occur in NASH to stimulate the activation of hepatic stellate cells with accumulation of fibrosis. However, patients will always present with various stages of advanced fibrosis, and agents that can reverse established fibrosis could reduce mortality and the need for liver transplantation. Simtuzumab (GS-6624, Gilead Sciences) was developed as an inhibitor of collagen cross-linking and maturation. However, it failed to meet key endpoints in a large clinical trial,[46] thus highlighting the challenges of this therapeutic approach. The galectin-3 inhibitor GR-MD-02 (Galectin Therapeutics), a complex carbohydrate that is administered intravenously with the goal of interfering with stellate cell activation, has been investigated. A clinical trial of this agent demonstrated measurable reductions in the hepatic venous pressure gradient from the portal vein to the hepatic vein in patients without varices.[47] This agent will likely be evaluated further in clinical trials.

Summary

NASH has become a major cause of liver disease in adults and children. Focusing on healthy eating habits and engaging in regular exercise are the primary recommendations for preventing and reversing NASH. However, these methods are not always successful for a variety of reasons; thus, pharmacologic approaches are needed. The pathogenesis of NASH is thought to be related to an overload of carbohydrates and fatty acids to the liver resulting in hepatocyte stress, apoptosis, inflammation, and fibrogenesis that leads to cirrhosis. Blocking the pathways of this multistep process is now being evaluated in many early- and late-stage clinical trials of drugs that have the potential to beneficially alter the underlying metabolic abnormalities and the liver cell stress, inflammation, and fibrosis that characterize NASH. Multiple therapeutic agents for NASH may be available over the next 2 to 5 years, and understanding their mechanisms of action will help guide a personalized approach to the prevention and treatment of NASH.

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