Pathophysiological mechanisms involved in non-alcoholic steatohepatitis and novel potential therapeutic targets

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Abstract
Non-alcoholic fatty liver disease (NAFLD) is a major health care problem and represents the hepatic expression of the metabolic syndrome. NAFLD is classified as non-alcoholic fatty liver (NAFL) or simple steatosis, and non-alcoholic steatohepatitis (NASH). NASH is characterized by the presence of steatosis and inflammation with or without fibrosis. The physiopathology of NAFL and NASH and their progression to cirrhosis involve several parallel and interrelated mechanisms, such as, insulin resistance (IR), lipotoxicity, inflammation, oxidative stress, and recently the gut-liver axis interaction has been described. Incretin-based therapies could play a role in the treatment of NAFLD. Glucagon-like peptide-1 (GLP-1) is an intestinal mucosa-derived hormone which is secreted into the bloodstream in response to nutrient ingestion; it favors glucose-stimulated insulin secretion, inhibition of postprandial glucagon secretion and delayed gastric emptying. It also promotes weight loss and is involved in lipid metabolism. Once secreted, GLP-1 is quickly degraded by dipeptidyl peptidase-4 (DPP-4). Therefore, DPP-4 inhibitors are able to extend the activity of GLP-1. Currently, GLP-1 agonists and DPP-4 inhibitors represent attractive options for the treatment of NAFLD and NASH. The modulation of lipid and glucose metabolism through nuclear receptors, such as the farnesoid X receptor, also constitutes an attractive therapeutic target. Obeticholic acid is a potent activator of the farnesoid X nuclear receptor and reduces liver fat content and fibrosis in animal models. Ursodeoxycholic acid (UDCA) is a hydrophilic bile acid with immunomodulatory, anti-inflammatory, antioxidative, antiapoptotic, antioxidant and antifibrotic properties. UDCA can improve IR and modulate lipid metabolism through its interaction with nuclear receptors such as, TGR5, farnesoid X receptor-α, or the small heterodimeric partner. Finally, pharmacologic modulation of the gut microbiota could have a role in the therapy of NAFLD and NASH. Probiotics prevent bacterial translocation and epithelial invasion, inhibit mucosal adherence by bacteria, and stimulate host immunity. In animal models, probiotics prevent obesity, decrease transaminase levels, and improve IR and liver histology in NASH.

Key words: Insulin resistance; Lipotoxicity; Non-alcoholic steatohepatitis; Physiopathology; Therapeutic targets
INTRODUCTION

Currently, non-alcoholic fatty liver disease (NAFLD) is recognized as a major health care problem, and it is the most common cause of raised transaminases in western countries, where it is considered the first cause of liver disease. Worldwide, its incidence and prevalence are increasing[1-4]. By 2020, non-alcoholic steatohepatitis (NASH) could be the main cause of liver transplantation[5].

NAFLD is characterized by fat accumulation, mainly as triglycerides, in the hepatocytes; it is associated with clinical factors such as obesity, dyslipidemia, and diabetes; its diagnosis requires the exclusion of other conditions that could be associated with steatosis, such as, significant alcohol consumption, viral hepatitis, use of steatogenic medications or hereditary disorders[6].

Histologically, NAFLD is classified as non-alcoholic fatty liver (NAFL) or simple steatosis, and NASH. NASH is characterized by the presence of steatosis accompanied by inflammatory infiltrate, ballooning of hepatocytes, and the presence of Mallory-Denk bodies; any degree of fibrosis can be present, but this is not a mandatory finding. Patients with NASH, mainly those with advanced fibrosis, are at higher risk for developing decompensated cirrhosis, hepatocellular carcinoma[7,8], or even death due to cardiovascular disease as a result of early atherosclerosis[9].

NAFLD represents the hepatic expression of the metabolic syndrome, and its pathophysiology involves several mechanisms, such as, glucose intolerance, insulin resistance (IR)[10,11], enhanced lipogenesis and lipotoxicity[12,13], hepatic and systemic inflammation[14], and oxidative stress[15].

The “multi-parallel hits” hypothesis is the most accepted for understanding the pathogenesis of NAFL and NASH and their progression to cirrhosis. This hypothesis proposes that many simultaneous hits derived from the gut and adipose tissue may promote inflammation and liver injury[13].

Unhealthy lifestyles are clearly related to NAFL and NASH. Excess energy intake through a diet rich in fat and carbohydrates[14-17] leads to failure of adipocytes to adapt in terms of proliferation and differentiation[18]. In the liver, free fatty acids (FFAs) are the main source for the synthesis of triglycerides. Similarly, excess dietary fat and de novo lipogenesis are two main factors contributing to the production of diacylglycerol and lysophosphatidyl choline, two non-triglyceride metabolites, which are responsible for lipotoxicity[19,20]. FFAs and cholesterol can also accumulate in the mitochondria leading to inflammation and liver injury mediated by tumor necrosis factor alpha and reactive oxygen species[12-21].

As NAFL and NASH are frequently associated with overweight, an important objective in the treatment of NAFL and NASH is to encourage weight loss; this can be achieved through lifestyle modification including a hypocaloric diet and/or aerobic exercise. The loss of at least 5% of body weight is necessary to improve steatosis, but a loss greater than 10% may be needed to improve steatohepatitis[22].

Pharmacological agents that could be useful in NAFL and NASH include glucagon-like peptide-1 (GLP-1) agonists. GLP-1 is an intestinal mucosa-derived hormone which is secreted into the bloodstream in response to nutrient ingestion; it favors glucose-stimulated insulin secretion, inhibition of postprandial glucagon secretion and delayed gastric emptying[23]. GLP-1 agonists also promote weight loss. In one study, treatment with liroglutide 1.2 mg once daily for 12 wk improved eating behavior in obese women with polycystic ovary syndrome (PCOS) and resulted in an average weight loss of 3.8 ± 0.1 kg (P < 0.001)[24]. In another study, short-term combined treatment with liroglutide 1.2 mg once daily and metformin 1000 mg twice daily was associated with significant weight loss and a decrease in waist circumference in obese women with PCOS who had previously been poor responders to metformin monotherapy[25]. In a cohort of obese nondiabetic women, short-term treatment with exenatide was also associated with a modest weight loss and decreased waist circumference[26].

GLP-1 is also involved in lipid metabolism; studies in animal models and in diabetic patients have found that GLP-1 agonists suppress postprandial elevations in lipids and lipoproteins[28,30]; result in a decrease in serum triglycerides, total cholesterol, low density lipoprotein-cholesterol and serum high density lipoprotein-cholesterol levels and reduce the development of atherosclerosis[31-34]. In mice, treatment with GLP-1 agonists was related to a reduction in the hepatic content of triglycerides[35]. Besides its property of enhancing...
insulin sensitivity, other possible mechanisms through which GLP-1 agonists may improve the lipid profile and metabolism are: Activation of peroxisome proliferator-activated receptor-\(\alpha\) on the hepatic cell surface, which reduces the synthesis of apolipoprotein C, degrades fat in plasma, and removes triglycerides\(^{[36-39]}\).

Once secreted, GLP-1 is quickly degraded by dipeptidyl peptidase-4 (DPP-4). DPP-4 inhibitors prolong the activity of incretins, GLP-1 and glucose-dependent insulino tropic polypeptide\(^{[24]}\). As the receptor for GLP-1 has been shown to exist in various cells, including hepatocytes\(^{[40,41]}\), DPP-4 inhibitors may have pleiotropic effects independent of lowering plasma glucose level and stimulating insulin secretion. In a retrospective study which included diabetic patients who received treatment with DPP-4 inhibitors and with abnormal transaminase levels, Kanazawa et al\(^{[42]}\) found that transaminase levels decreased after six months of treatment with DPP-4 inhibitors.

Ursodeoxycholic acid (UDCA) is not approved for treating NASH; however, it is a hydrophilic bile acid with immunomodulatory, anti-inflammatory, anti-apoptotic, antioxidant and anti-fibrotic properties. It also reduces the mitochondrial membrane permeability and the release of hydrolytic enzymes from damaged hepatocytes. In a recent study which included patients with biopsy-proven NASH, high-dose UDCA for 12 mo reduced transaminase levels, the degree of fibrosis, and improved IR independently of the change in body weight. UDCA modulated lipid and glucose metabolism through its interaction with nuclear receptors such as, TGR5, farnesoid X receptor-\(\alpha\), or the small heterodimeric partner\(^{[43]}\).

Obeticholic acid is a potent activator of the farnesoid \(X\) nuclear receptor that reduces liver fat content and fibrosis in animal models of NAFLD. In a multicenter, double-blind, placebo-controlled, randomized clinical trial, treatment with obeticholic acid in adult patients with NASH for 72 wk improved the histological features of NASH, but its long-term benefits and safety require further study\(^{[44]}\).

Recently, it was demonstrated that the gut-liver axis plays a key role in the pathogenesis of obesity, NAFL, NASH and their progression. The gut microbiota is composed of bacteria, viruses, yeasts and parasites. Gut microbes are able to interact actively with the host immune system modulating inflammation, IR, intestinal permeability, and endotoxemia\(^{[45]}\). Pharmacologic modulation of the gut microbiota could have a role in the therapy of NAFL and NASH. Probiotics prevent bacterial translocation and epithelial invasion, inhibit mucosal adherence by bacteria, and stimulate host immunity. In animal models, probiotics also prevent obesity, improve IR and liver histology in NASH, and decrease transaminase levels\(^{[46-50]}\). In patients with NAFLD the use of probiotics improved transaminases, the cytokine profile and oxidative stress\(^{[51]}\).

In summary, NAFLD is an important health care problem. The pathophysiology of NAFL and NASH and their progression involve multifactorial and complex processes, where multi-parallel simultaneous hits derived from the gut and adipose tissue promote a pro-inflammatory response and liver injury. All of these represent attractive therapeutic targets. Pharmacological agents such as GLP-1 agonists, DPP-4 inhibitors, UDCA, obeticholic acid and probiotics need to be explored in clinical trials specifically for treating NAFL and NASH.

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