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Review

Recent insights on the role of cholesterol in non-alcoholic fatty liver disease

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ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) encompasses a spectrum of hepatic histopathological changes ranging from non-inflammatory intracellular fat deposition to non-alcoholic steatohepatitis (NASH), which may progress into hepatic fibrosis, cirrhosis, or hepatocellular carcinoma. NAFLD hallmark is the excessive hepatic accumulation of neutral lipids that result from an imbalance between lipid availability and lipid removal. Recent data suggest that disturbed hepatic cholesterol homeostasis and liver free cholesterol (FC) accumulation are relevant to the pathogenesis of NAFLD/NASH. Hepatic FC accumulation in NAFLD results from alterations in intracellular cholesterol transport and from unbalanced cellular cholesterol homeostasis characterized by activation of cholesterol biosynthetic pathways, increased cholesterol de-esterification and attenuation of cholesterol export and bile acid synthesis pathways. FC accumulation leads to liver injury through the activation of intracellular signaling pathways in Kupffer cells (KCs), Stellate cells (HSCs) and hepatocytes. The activation of KC and HSC promotes inflammation and fibrogenesis. In addition, FC accumulation in liver mitochondria induces mitochondrial dysfunction, which results in increasing production of reactive oxygen species, and triggers the unfolded protein response in the endoplasmic reticulum (ER) causing ER stress and apoptosis. These events create a vicious circle that contributes to the maintenance of steatosis and promotes ongoing hepatocyte death and liver damage, which in turn may translate into disease progression. In the present review we summarize the current knowledge on dysregulated cholesterol homeostasis in NAFLD and examine the cellular mechanisms of hepatic FC toxicity and its contribution to ongoing liver injury in this disease. The therapeutic implications of this knowledge are also discussed.

1. Introduction

1.1. Concept, clinical spectrum and pathogenesis of non-alcoholic fatty liver disease (NAFLD)

The acronym non-alcoholic fatty liver disease (NAFLD) is an umbrella term used to describe a clinicopathological entity defined by the presence of a spectrum of hepatic histopathological changes ranging from non-inflammatory intracellular fat deposition (isolated steatosis) to non-alcoholic steatohepatitis (NASH), with the latter being characterized by steatosis, necro-inflammatory changes and various degrees of liver fibrosis [1,2]. Currently, NAFLD is recognized as the most common form of liver disease worldwide affecting between 25 and 30% of the general population [3,4]. Notably, NAFLD is highly prevalent among overweight and obese patients and in subjects with type 2 diabetes (T2DM) [5,6]. Moreover, NAFLD is strongly associated with the features of metabolic syndrome, and the hepatic manifestation of this cluster of features is linked to increased cardiometabolic risk [7,8]. A clinically relevant aspect of NAFLD is that a variable proportion of patients, mainly those with NASH, exhibit an increased liver-related mortality due to the progression to cirrhosis and its associated complications, including hepatocellular carcinoma [9]. In fact, recent data shows that NASH is expected to become the leading cause of liver transplantation by 2020 [10]. Finally, NAFLD has been also linked to an increased risk of incident T2DM and cardiovascular disease [9,11].

Aspects related to the transition from isolated steatosis to NASH, the progressive form of the disease, are key issues in the study of NAFLD. However, factors involved in the development of more aggressive forms of the disease remain only partially unveiled [12]. Recognition of these factors is important for the identification of potentially useful therapeutic targets [13,14]. In addition to well-known factors involved in NASH pathogenesis and progression (i.e., degree of obesity, magnitude of insulin resistance (IR), adipokine imbalance, excessive dietary fructose intake and ongoing oxidative stress driven by lipotoxic metabolites), emerging experimental and human data suggest that disturbed hepatic cholesterol homeostasis and free cholesterol (FC) accumulation are relevant to the pathogenesis of NASH [15,16]. In the present review,

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we summarize the currently available knowledge about cellular mechanisms of cholesterol toxicity, and we examine how alterations in cholesterol homeostasis may promote hepatic cholesterol overload and contribute to ongoing liver injury in NAFLD.

1.2. Pathogenesis of NAFLD/NASH

The precise pathophysiology of NAFLD is not fully understood [17]. Current concepts on disease pathogenesis are evolving as new information is generated from both experimental models and human studies [12]. Conceptually, NAFLD is generally conceived as a sequential process consisting first in steatosis development [i.e., pathological accretion of lipids in the form of triglycerides (TGs)]. In some individuals, steatosis may promote the occurrence of hepatocyte injury and death and subsequently induce localized inflammation and trigger a stereotyped hepatic fibrogenic response to injury [18,19]. The latter fuels the progression to advanced fibrosis and cirrhosis [12,20]. Thus, key aspects of NAFLD pathogenesis include the mechanisms of fat deposition in the liver cells and the determinants of necro-inflammatory and fibrotic changes, that can drive disease progression [21]. Steatosis development is clearly related to the occurrence of IR at the level of the hepatic, muscle and adipose tissues [22,23]. Although the majority of NAFLD patients exhibit systemic IR it is difficult to determine which of the insulin sensitive tissues is the primary site of IR in NAFLD [24]. This is a complex issue that faces methodological problems to be solved since the precise assessment of insulin resistance requires complex techniques such as the euglycemic hyperinsulinemic clamp and the use of stable isotopes, which is cumbersome [25]. Some recent lines of evidence suggest that hepatic IR, secondary to the accumulation of diacylglycerols (DAGs), may be the primary pathogenic event [26], although this hypothesis remains controversial [24]. The observation that the disease may recur after transplantation [27] suggests that peripheral IR is a primary event. Based on this observation and additional existing data, the predominant view about steatosis development is that an initial impaired peripheral insulin action in white adipose tissue (WAT) would lead to an uninhibited lipolysis that, in turn, produces an increased flux of fatty acids (FAs) to the liver and other organs. Visceral WAT is considered an important contributing source to the overflow of FA to the liver due to the direct drain of its circulation to the portal vein [20,28] but the contribution of subcutaneous fat is also substantial due to the size of this depot particularly in obese subjects [29]. Lipolysis in adipocytes is a complex and tightly regulated phenomenon that involves neuroendocrine signals resulting in the activation of lipolytic enzymes that drive lipid mobilization. Among these enzymes, hormone sensitive lipase (HSL) and adipocyte triacylglycerol lipase are the key enzymes for lipolysis initiation [30]. Details of regulatory mechanisms of adipocyte lipolysis are beyond the scope of the present review and can be found elsewhere [31,32]. Suffice to say that insulin by regulating HSL limits the liberation of FA from WAT and rather induces de novo lipogenesis in this tissue. Thus, if IR is present lipolysis becomes hyperactivated in adipocytes, resulting in an increased release of FA to plasma.

The reasons of IR development in WAT are complex and involve a sequence of events that determine a phenomenon known as adipose tissue dysfunction that in turn determines local IR and dysregulation of other physiological functions of WAT such as the secretion of adipokines [33]. Briefly, in conditions of excessive calorie intake, adipocytes become hyperplastic, which is associated to a relative local hypoxia and the development of WAT stress and autophagy followed by the occurrence of immune cell infiltration and inflammation [33,34]. This dysfunctional and injured WAT become resistant to insulin action and uninhibited lipolysis occurs. Dysregulated adipokine secretion is characterized by the secretion of a proinflammatory and atherogenic adipokine profile with decreased adiponectin (an insulin-sensitive adipokine) and increased tumor necrosis factor-α (TNF-α, a cytokine that promotes IR both locally and systemically) [33,35]. Thus, uninhibited lipolysis of triacylglycerol in WAT determines an overflow of nonesterified FA to the liver. In fact, it has been estimated that approximately 60% of TG present in the liver in patients with NAFLD originate from WAT [36,37]. Uptake of FA from plasma in the liver is mediated by fatty acid transport proteins (FATPs) and the fatty acid translocase FAT/CD36. Both FATP2 and FATP5 are highly expressed in hepatocytes and FATP5 knockout mice are protected against liver fat accumulation. However, no evidence of a relevant role in humans is available [38]. On the other hand, FAT/CD36 has been found upregulated in both human and experimental NAFLD [39,40], which may contribute to liver fat overload. Once inside the liver FA is bound by cytosolic fatty acid-binding proteins (FABPs). Mice deficient in FABP are resistant to diet-induced hepatic steatosis [41] but the role of these proteins in human NAFLD is not known.

In addition to the overflow of FA to the liver from WAT, enhanced de novo hepatic lipogenesis is also recognized as an important contributor to TG accumulation in liver cells [42,43]. Studies using isotope labeling have shown that this pathway accounts for 25% of hepatic TG in NAFLD patients [36,37]. Increased de novo lipogenesis is driven by compensatory hyperinsulinemia present in IR states [42]. In fact, insulin increases the hepatic activity of critical transcription factors, such as sterol regulatory element-binding protein-1c (SREBP-1c), carbohydrate response element-binding protein (ChREBP) and peroxisome proliferator-activated receptor (PPAR)-γ, which are the major drivers of hepatic de novo lipogenesis [42,44]. Stimulation of this pathway by dietary components, such as fructose and high-fructose corn syrup, has been proposed as a contributing factor to the development and severity of NAFLD [45]. However, this idea has been disputed by other studies showing that the reported associations of fructose with clinical or histopathological evidence of NAFLD may be more attributable to excess energy than fructose itself [46,47].

The precise role of additional pathways of FA metabolism in NAFLD, such as impaired hepatic FA oxidation (which would lead to a reduction in FA utilization) and/or impaired synthesis or secretion of very low-density lipoprotein (VLDL) (which would lead to a decrease in the export of TG from the liver), is still not well defined. In the case of FA oxidation although data support the presence of mitochondrial dysfunction in NAFLD, data on the role of impaired of FA oxidation in this setting is inconclusive [48]. With regard to dysregulation of VLDL production and secretion, it has been shown that individuals with NAFLD exhibit and increase in VLDL secretion [49] likely as a compensatory response to intracellular TG accumulation. However, the increase in hepatic TG export through this pathway seems to be insufficient to normalize the elevated hepatic TG content.

Once steatosis is established, liver cell damage may occur with accompanying inflammation and/or fibrosis consistent with NASH [19,20,50]. Multiple factors, including worsening IR, insults from the gut [bacteria derived products that can activate Toll-like receptors (TLRs)] in both hepatocytes and resident macrophages and/or the adipose tissue (saturated FA-induced lipotoxicity), promote hepatic cell injury and death through a variety of mechanisms [51–53]. Although in some cases steatosis precedes NASH, it is also possible that steatosis develops with other features of NASH simultaneously. In fact, it has been proposed that subjects with bland steatosis and NASH could belong to different subsets of individuals with separate histological features and pathophysiology [54–56].

1.3. Modulators of liver injury in NAFLD

Factors that trigger or modulate liver damage in the setting of NAFLD can help to identify potentially useful therapeutic targets [13,14]. Indeed, correction and management of known NASH-related factors involved in pathogenesis and progression, such as the degree of obesity, the severity of IR, the coexistence of T2DM, the occurrence of oxidative stress and excessive dietary energy and fructose intake, are the current focus of NAFLD/NASH treatment [14,57,58]. In addition, identification of genetic factors that determine risk of disease occurrence or progression may help to identify individuals who may experience associated

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