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Review

Oxidized LDL at the crossroads of immunity in non-alcoholic steatohepatitis

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ABSTRACT

Non-alcoholic steatohepatitis (NASH) is viewed as the hepatic manifestation of the metabolic syndrome and is a condition hallmarked by lipid accumulation in the liver (steatosis) along with inflammation (hepatitis). Currently, the etiology and mechanisms leading to obesity-induced hepatic inflammation are not clear and, as a consequence, strategies to diagnose or treat NASH in an accurate manner do not exist. In the current review, we put forward the concept of oxidized lipids as a significant risk factor for NASH. We will focus on the contribution of the different types of oxidized lipids as part of the oxidized low-density lipoprotein (oxLDL) to the hepatic inflammatory response. Furthermore, we will elaborate on the underlying mechanisms linking oxLDL to inflammatory responses in the liver and on how these cascades can be used as therapeutic targets to combat NASH. This article is part of a Special Issue entitled: Lipid modification and lipid peroxidation products in innate immunity and inflammation edited by Christoph J. Binder.

1. Introduction

In parallel to the steep increase of obesity and type 2 diabetes, non-alcoholic fatty liver disease (NAFLD) is currently the most common liver disease worldwide [1] and increasingly recognized as the hepatic manifestation of the metabolic syndrome (MetS) [2,3]. NAFLD comprises a spectrum of liver disorders ranging from simple lipid accumulation in the liver (steatosis) to fibrosis and cirrhosis, which will eventually lead to liver failure and death [4]. A key step in developing such advanced liver diseases is the progression of steatosis to non-alcoholic steatohepatitis (NASH), which is characterized by the combination of steatosis and inflammation in the liver [5]. Furthermore, the presence of fibrosis in the liver is nowadays considered as a key symptom of active NASH, which is characterized by diminished long-term prognosis [6].

As NAFLD is closely related to the presence of obesity and MetS, aberrations in lipid metabolism are one of the major hallmarks of this disease [7]. Observed lipid deviations in NAFLD patients include elevated levels of plasma and liver triglycerides (TGs), low levels of plasma high-density lipoproteins (HDL) and elevated low-density lipoproteins (LDL) and total cholesterol levels [4,8,9]. Apart from abnormalities in lipid metabolism, another important aspect of NASH is the presence of oxidative stress [10]. The increased generation of reactive oxygen species (ROS) combined with an impaired antioxidant defense creates a condition which is referred to as oxidative stress. Indeed, molecular components associated with oxidative stress have been demonstrated to be highly reactive with surrounding tissues, inducing tissue injury [11].

Considering that disturbances in lipid metabolism and oxidative stress make up two important aspects of NASH, increasing evidence indicates an important role for oxLDL-derived oxidized lipids in the pathogenesis of NASH. In this review, we aim to give an overview of the current data linking oxidized lipids to NASH and explain the mechanisms that lead to and are affected by lipid oxidation. Additionally,

Abbreviations: 25HC, 25-hydroxycholesterol; 27HC, 27-hydroxycholesterol; 4-HNE, 4-hydroxynonenal; CASP-1, Caspase-1; CD36, cluster of differentiation 36; Col1a1, collagen type 1, alpha 1; CONV-R, conventionally raised; CVD, cardiovascular disease; FA, fatty acid; FXR, farnesoid X receptor; FFA, free fatty acids; FMT, fecal microbiota transplantation; GF, germ-free; GSH, glutathione; Gsr, glutathione reductase; HDL, high-density lipoprotein; HSC, hepatic stellate cell; HFD, high fat diet; KC, kupffer cell; LDL, low-density lipoprotein; LOX-1, lectin-like oxLDL receptor 1; LOXL2, lysyl oxidase-like-2; LSEC, liver sinusoidal endothelial cell; MAA, malondialdehyde-acetaldehyde; MDA, malondialdehyde; MetS, metabolic syndrome; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; NLRP3, NACHT, LRR and PYD domains-containing protein 3; OCA, obeticholic acid; OSE, oxidation-specific epitope; oxLDL, oxidized LDL; PC, phosphorylcholine; PCSK9, pro-protein convertase subtilisin/kexin type 9; POP, plant sterol oxidation product; PUFA, polyunsaturated fatty acid; ROS, reactive oxygen species; SdLDL, small-dense LDL; Sesns, Sestrins; SR, scavenger receptor; SR-A, scavenger receptor A; TG, triglyceride; Tgf-β, transforming growth factor beta; TXNIP, thioredoxin-interacting protein.

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we will discuss and evaluate the potential clinical benefits of targeting oxidized lipids in NASH.

2. Liver lipids and related oxidation processes

Different types of lipids are proposed to influence the disease spectrum of NAFLD. First, the accumulation of TGs determines the histological appearance of a steatotic liver. Structurally, TGs are created by the fusion of a glycerol backbone and three free fatty acids (FFAs) that attach to it (Fig. 1A). Therefore, increases in hepatic TG levels can be derived from an elevated influx of FFAs into the liver from lipolysis of adipose tissue, increased hepatic synthesis, reduced lipid export from the liver and an excess dietary fat intake [12]. Second, in spite of the abundant presence of TGs (and as such fatty acids (FAs)) in the steatotic liver, it becomes evident that not triglycerides, but rather cholesterol is an important lipid compound in NAFLD progression. Putting mice on a high-cholesterol diet was shown to induce hepatic inflammation while omitting cholesterol from the diet prevented the appearance of hepatic inflammation [13–15]. Moreover, also in humans specifically the cholesterol levels were found to associate with the level of hepatic inflammation [16,17]. Together, these findings indicate that cholesterol is a significant risk factor in the onset of NASH.

Relevantly, hepatic accumulation of FFA has been associated with disturbances of cellular processes that are linked to inflammation such as protein unfolding [18], innate immunity [19,20], destabilization of lysosomal membranes [21], oxidative stress [22] and mitochondrial function [23]. In contrast, it has been shown that accumulation of lipid compounds *per se* does not induce hepatic inflammation. Moreover, several findings even suggested that TG synthesis is protecting the liver from lipotoxicity by buffering FFA accumulation [24,25]. Furthermore, authors have also argued the role of FAs in inducing hepatic lipotoxicity in NASH [26,27]. Similarly, lipid-reducing drugs were shown not to improve NASH [28,29]. Together, despite the strong correlation between dyslipidemia and NASH, it has become clear that disturbances in lipid metabolism cannot merely explain the progression towards the inflammatory response in the liver.

Oxidative stress is an important process which has been shown to be involved in the progression from a benign fatty liver to NASH [30,31]. Elevated ROS levels can be derived from a range of stimuli, including cellular stress, mitochondrial dysfunction, disturbances in antioxidants and other enzyme dysfunctions [32]. Next to its physiological role in the regulation of signaling cascades, ROS are recognized as oxidizing agents that impair the structure of important components of the cell such as DNA, lipid membranes and proteins [33]. Therefore, given that both lipid metabolism and oxidative stress in the liver make up important aspects of NASH pathogenesis, it is not surprising that both processes are intertwined, creating inflammatory oxidized lipids affecting the liver.

One of the best-studied examples of an oxidized lipoprotein associated with inflammation is oxidized LDL (oxLDL). Though initially associated with atherosclerosis [34], oxLDL has recently been associated with inflammatory processes in NASH [35,36]. In both the arteries and the liver, the uptake of oxLDL by macrophages was shown to be an important player in generating a detrimental inflammatory response [37].

Concerning oxLDL, an important footnote should be stated. Initially, oxLDL was described as an LDL particle containing protein components that are modified due to oxidation reactions. As a result of these reactions, a net negative charge could be observed on this particle, making it prone to recognition by macrophages [38,39]. However, it became clear that in contrast to the protein modifications, it is more likely that the oxidation of lipids, present inside the lipoprotein particle, determines the pro-inflammatory phenotype observed in oxLDL-loaded macrophages [40]. Firstly, phospholipid oxidation products were shown to act pro-inflammatory [41]. Next, fatty acids, both in the ester and free form, are prone to interact with free radicals (Fig. 1A). However, despite the formation of the highly reactive malondialdehyde (MDA) product (Fig. 1A), oxidation of polyunsaturated fatty acids (PUFAs) is considered as a protective mechanism to prevent oxidation of sterol-like structures, then referred to as cholesterol oxidation products or oxycholesterols [42]. Indeed, ROS-induced oxidation of sterol-like structures has been associated with inflammatory processes in the liver, arteries and central nervous system [43–45].

Of note, a clear distinction (both structurally and biologically) has to be made between ROS-mediated oxidation and enzymatic oxidation of cholesterol. ROS-mediated oxidation (or autoxidation) of cholesterol induces ring-oxidation products, which are generally considered to be created in a non-enzymatic manner (Fig. 1B, *upper panel*). In contrast, cholesterol also undergoes enzymatic oxidation, thereby creating side-chain oxidation products (Fig. 1B, *lower panel*). This hydroxylation reaction is mediated by cytochrome P450 enzymes and is necessary to create the intermediates for bile acids and steroid hormones [46]. Also, in contrast to the pro-inflammatory role of ROS-generated products, cholesterol oxidation products generated via enzymatic reactions were shown to act anti-inflammatory. Via subcutaneous injections into a hyperlipidemic mouse model, 27-hydroxycholesterol (27HC), an intermediate in bile acid synthesis, was shown to reduce hepatic inflammation [47]. Additionally, hematopoietic overexpression of CYP27, the enzyme which generates 27HC, led to a reduction in hepatic inflammation independent of plasma 27HC levels [48]. Furthermore, 25-hydroxycholesterol (25HC) was recently shown to decrease macrophage-induced inflammation via regulating inflammasome activation [49]. Finally, estrogens have also been linked to anti-inflammatory immune responses [50].

Compounds that are structurally and functionally similar to cholesterol (and therefore also subject to oxidation reactions) are plant sterols. Via incorporation in oils, cereals, fruits and nuts (among others), plant sterols make up about 20% of the total amount of sterols consumed in a Western-type diet, equaling 300 mg/day [51]. Relevantly, though plant sterols cannot be synthesized from any precursor inside the body [52], important biological functions have been assigned to these steroid complexes such as their blood cholesterol-lowering potential and their ability to reduce cardiovascular risk [53,54]. However, analogous to cholesterol, plant sterols are also prone to undergo oxidation reactions, leading to the generation of plant sterol oxidation products (POPs) or oxyphytosterols [55]. The formation of these oxyphytosterols can be achieved by uptake from the diet or via enzymatic reactions or radical autoxidation of absorbed plant sterols, with the latter being the main source [56]. Several inferential data have suggested the contribution of POPs to inflammatory processes in disorders such as atherosclerosis [57]. However, due to the lack of knowledge concerning oxyphytosterol metabolism and inconclusive results obtained with them, the exact effects of POPs on inflammatory responses *in vivo* and in humans is still under debate [53]. Despite mainly being linked to cardiovascular disorders, well-designed studies demonstrated the accumulation of oxyphytosterols in a variety of tissues with the liver accumulating the highest concentration of these oxidized lipids [58,59]. Therefore, regardless of their unknown functions, oxyphytosterols might contribute to inflammatory processes in the liver, which justifies further research of these compounds in the context of NASH.

Of relevance, though both intra- and extracellular-derived oxidized lipids contribute to the inflammatory response in the liver, their relative contribution has, to our knowledge, not been investigated yet. However, crosstalk between intra- and extracellular oxidation mechanisms has been implicated [60], thereby implying that both intra- and extracellular oxidized lipids likely assist each other in mediating inflammatory processes. In summary, it is important to identify which lipids have undergone which type of oxidation reaction in order to assess the possible associated detrimental effects.

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