Review Article

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Metalloproteinases in non-alcoholic fatty liver disease and their behavior in liver fibrosis

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Abstract:
Non-alcoholic fatty liver disease (NAFLD) is a clinical entity of high prevalence in the world characterized by fatty infiltration of liver tissue in the absence of alcohol consumption. The natural history of the disease develops in successive phases reflected in different histological stages, with 10–20% of patients developing liver cirrhosis and fibrosis. Fibrosis is a basic connective tissue lesion defined by the increase of the fibrillary extracellular matrix (ECM) components in a tissue or organ. Matrix metalloproteinases (MMPs) constitute a family of endopeptidases, which are involved in ECM and basement membranes components degradation. Fibrogenic process is characterized by altered ECM composition, associated with modifications in MMPs behavior. The active cross-talk between adipose tissue and liver can be altered in pathologies associated to insulin resistance (IR), such as NAFLD. The role of adipokines on MMPs behavior in the liver could be partly responsible of liver damage during IR. The aim of this revision is to describe the behavior of MMPs in NAFLD and its role in the associated fibrosis.

Keywords: adipocytokines, fibrosis, metalloproteinases, non-alcoholic fatty liver

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Introduction

Non-alcoholic fatty liver disease (NAFLD) is a clinical entity of high prevalence in the world (10–40%), characterized by fatty infiltration of liver tissue (>5% of hepatocytes) in the absence of alcohol consumption. It clusters a morphological spectrum of disorders ranging from simple triglycerides (TG) accumulation in hepatocytes (hepatic steatosis; HS) to other entities of worse prognosis like non-alcoholic steatohepatitis (NASH), characterized by steatosis, lobular inflammation and hepatocellular ballooning injuring. The natural history of the disease develops in successive phases reflected in different histological stages, with 10–20% of patients developing liver cirrhosis and fibrosis [1]. Parenchymal and non-parenchymal cells that constitute the functional architecture of the liver are supported by an extracellular matrix (ECM) composed of fibrous proteins and proteoglycans. The behavior and composition of ECM modulates the morphological organization and physiological function of the liver thus, dysregulation of ECM homeostasis is associated with degenerative liver diseases. The maintenance of the composition and function of the ECM is regulated by a family of zinc-dependent endopeptidases called metalloproteinases (MMPs) [2].

NAFLD is considered the hepatic manifestation of metabolic syndrome (MS) and is closely related to insulin resistance (IR) and cardiovascular disease (CVD) [3]. In the recent years, NAFLD has emerged as the most prevalent chronic liver disease, due to the global obesity epidemic [4]. NAFLD results from complex interactions between genetic, hormonal and nutritional factors, being obesity and MS the most important risk factors identified until now [5]. The active cross-talk between adipose tissue and liver can be altered in these situations. It is well known that the increase in circulating proinflammatory adipokines promotes liver disease. The role

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of these adipokines on MMPs behavior in the liver could be partly responsible for liver damage during IR and obesity.

The aim of this revision is to describe the behavior of MMPs in NAFLD and its role in the associated fibrosis.

Metalloproteinases

MMPs constitute a family of more than 25 zinc-dependent endopeptidases, which are involved in ECM and basement membranes components' degradation [2]. The ECM is a multimolecular complex structure comprising collagen and elastin fibers, structural glycoproteins including fibronectin and laminin, and mucopolysaccharides. It is organized into a three-dimensional (3D) network and in physiological states a balance between synthesis, deposit and degradation of ECM components exist [6]. According to their substrate specificity, MMPs can be divided into five major groups: collagenases, stromelysins, matrilysins, gelatinases and membrane-type MMPs [7]. These enzymes are expressed in different cell types including fibroblasts, neutrophils, monocytes, macrophages and endothelial cells. MMPs are multidomain enzymes that have several similar structural domains including cysteine-switch motif (PRCGXPD) in their propeptide, which maintain MMPs in the zymogen form (pro-MMPs), the catalytic zinc-binding domain (HEXGHXXGXXHS) and the hemopexin-like domain [8]. MMPs are regulated at different levels such as gene transcription, zymogen activation and enzyme secretion as well as by endogenous specific tissue inhibitors (TIMPs) [2]. Some MMPs are expressed constitutively, while others are only expressed under specific stimuli, such as reactive oxygen species (ROS), pro-inflammatory cytokines [interleukin (IL-1) and tumor necrosis factor alpha (TNF-α)] or by the contact of inflammatory cells with the collagen of the ECM. They are secreted as latent zymogens (pro-MMPs) which are inactive due to the interaction of the zinc ion in the catalytic domain with the sulfhydryl group of the Cys residue in the prodomain. Pro-MMPs are activated by the disruption of the cysteine switch by different mechanisms such as the proteolytic action of other proteases, conformational changes generated by nitric oxide (NO) and ROS, or by chaotropic agents and denaturants such as sodium dodecyl sulfate, low pH and heat treatment, among others. When this occurs the -SH group is replaced by H_2O and the enzyme gains the ability to hydrolyze the propeptide for its complete activation [9].

With reference to protein specific inhibitors, there are at least four members of TIMP family that form tight inhibitory 1:1 complexes with MMPs. The balance between activated MMPs and TIMPs determines the net result of MMPs activity in tissues. However, in pathological situations, this balance might be disrupted leading to MMPs uncontrolled activation [10].

Among the physiological processes, MMPs are involved in organogenesis, angiogenesis, apoptosis, cell proliferation and motility. Any disruption in MMPs regulation could lead to the development of diseases such as cancer, rheumatoid arthritis, atherosclerosis, obesity, fibrosis and endometriosis [11], [12].

Previous studies have reported the behavior of different MMPs in IR states in humans [13], [14] and animal models [15]. It has been observed that circulating MMPs are increased in patients with MS [13], [16] meanwhile controversial results have been described in in vitro studies and in animal models [15], [17].

Fibrosis in NAFLD

Fibrosis is a connective tissue lesion defined by the increase of the fibrillary ECM components. One-third of patients with NAFLD progress to liver fibrosis in 4–5 years after the first liver biopsy. It is important to mention that recent studies have reported that advanced fibrosis in NAFLD predicts not only liver-related mortality but also increased mortality due to cardiovascular events [18].

In healthy liver, hepatic stellate cells (HSCs), localized in the perisinusoidal space, are the most important source of ECM. Upon chronic damage to liver tissue, HSCs activate and differentiate into fibroblast-like phenotype, increasing ECM deposition. Mediators released from damaged hepatocytes, such as lipid peroxidation products and ROS, as well as activated Kupffer cells are important effectors driving HSC activation [19]. After chronic inflammatory processes, hepatic fibrosis develops leading eventually to cirrhosis or liver cancer. This process may result from an imbalance between enhanced matrix synthesis by HSCs and diminished breakdown of connective tissue proteins. The hepatic fibrogenesis process takes place in two phases, the first one is characterized by inflammation produced by fat accumulation in the liver as TG, increasing liver lipotoxicity. This process activates hepatocytes and recruits T-cells, while biliary epithelial cells activates Kupffer cells. The result is the increase in oxidative stress and the production of free radicals as well as other soluble factors, such as transforming growth factor beta (TGF-β), capable of stimulating HSCs. In the second fibrotic phase, quiescent

HSCs turn into myofibroblasts and lead to the apoptosis of hepatocytes, inducing the accumulation of fibrotic cells. These cells induce immune cells recruitment, which are responsible of chronic inflammation [19], [20].

The fibrogenic process is associated with MMPs/TIMPs imbalance, which causes excessive accumulation of ECM components [20].

MMPs and fibrosis

The fibrogenic process is characterized by ECM alterations, associated with MMPs/TIMPs imbalance [6]. In the human liver, different MMPs and TIMPs are expressed [19], being MMP-2 and MMP-9 that are of great importance in hepatic fibrosis. It has been demonstrated that variations in MMPs expression correlate with the fibrotic stage of liver diseases [21]. Until now, the behavior of MMPs in the liver fibrosis process is controversial. Previous studies reported increased collagenase activity in the early stage of liver fibrosis and reduced collagenase activity in advanced stages [22]. In our laboratory, we studied the behavior of circulating and hepatic MMP-2 and -9 activity in patients with biopsy-proven NAFLD. In NAFLD patients, MMP-2 plasma activity was increased in the severe fibrosis stage independently of the steatosis grade. However, liver MMP-2 activity was decreased compared to non to moderate fibrosis patients (Figure 1). Previously, Hemman et al. reported that in activated HSCs, TIMP-1 expression is increased and concomitantly MMPs activity is reduced, leading to protein accumulation [21]. Meanwhile, in human liver biopsies, no difference in MMP-9 activity was observed in steatosis situations. Wanninger et al. demonstrated that in human fatty liver MMP-9 activity is associated with hepatic inflammation and fibrosis but not with hepatic steatosis and body weight [23].

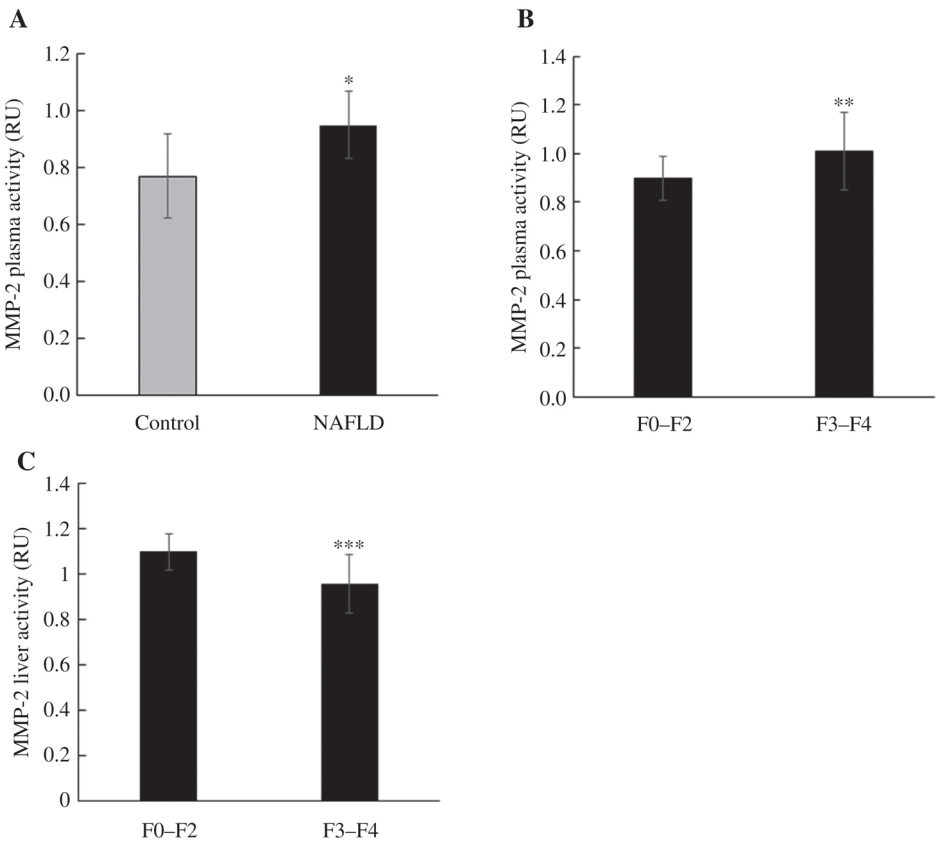

Figure 1: MMP-2 plasma and liver activity in (A) control and NAFLD group. $^{*}p < 0.001$. (B) Fibrosis stages in NAFLD group. $^{**}p < 0.049$. RU means relative units. (C) MMP-2 liver activity in non-to-moderate fibrosis (F0–F2) and severe fibrosis (F3–F4) group. $^{***}p = 0.024$.

Anyway, there are few studies analyzing the role of MMPs in NAFLD, and most of them studied mRNA levels, but even their expression pattern remains controversial.

IR is frequently linked with hepatic fibrosis; thus, hyperinsulinemia may condition MMPs behavior [24]. In individuals with IR without NAFLD, we have previously demonstrated an increase in circulating MMP-2 activity, associated with a more atherogenic lipid profile [13], [25]. Similar results were also observed in patients

with NAFLD, and positive associations between MMP-2 plasma activity and body mass index (BMI), waist circumference and atherogenic lipoprotein profile were found (Table 1).

Table 1: Associations of MMP-2 plasma activity and biochemical parameters.

	MMP-2	
	R	p-Value
BMI	0.330	0.004
WC	0.475	0.001
TG	0.341	0.002
LDL-chol	0.292	0.009
Non-HDL-chol	0.301	0.007
HDL-chol	−0.328	0.003
Adiponectin	−0.424	0.001

Spearman's or Pearson's tests according to data distribution. BMI, Body mass index; chol, cholesterol; TG, triglycerides; WC, waist circumference.

MMPs and adipocytokines in fibrosis

Adipocytokines are polypeptides synthesized and secreted by adipose tissue which exerts autocrine, paracrine and endocrine functions. Through these molecules adipose tissue directly connects with the liver, exerting modulatory effects. A continuous dynamic cross-talk between adipokines and liver exists, resulting in a beneficial or unfavorable final effect [26]. During the expansion of adipose tissue, the adipocytokines profile is modified, contributing to the development of hepatic steatosis and to the progression to NASH [27].

In IR and related disorders most of the proinflammatory adipocytokines, such as leptin, IL-6 and TNF-α, are upregulated, while anti-inflammatory adipocytokines levels, such as adiponectin, are decreased. This imbalance promotes susceptibility to metabolic and inflammatory diseases. Leptin and adiponectin are the main cytokines synthesized in adipose tissue and exhibit different functions and opposite effects on the liver [28] (Figure 2).

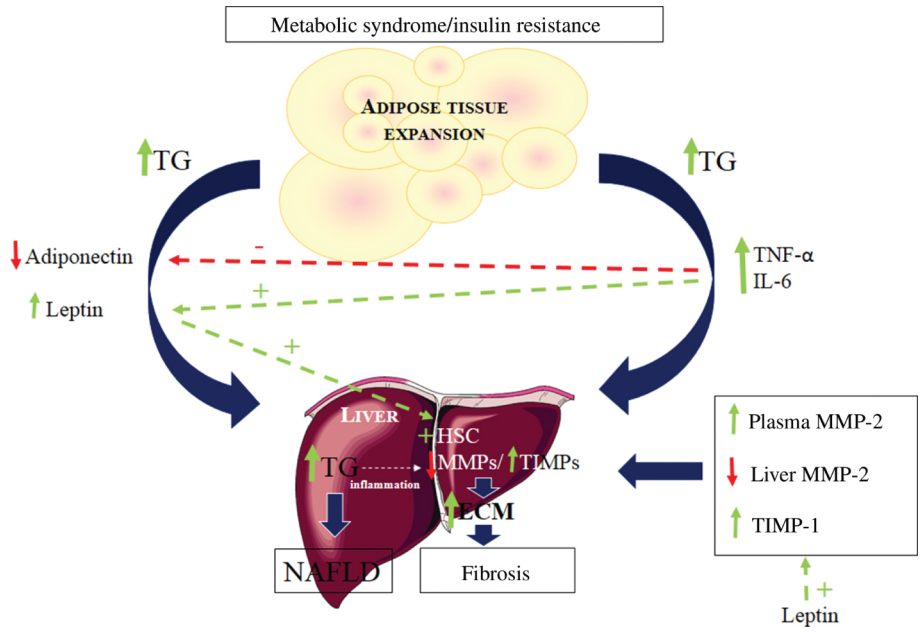

Figure 2: Role of metalloproteinases in the pathophysiology of non-alcoholic fatty liver disease and liver fibrosis in insulin resistance.
The increase in circulating proinflammatory adipocytokines and the decrease in adiponectin levels lead to an imbalance in metalloproteinases and their inhibitors, which contributes to the development of fibrosis. ECM, extracellular matrix; HSC, hepatic stellate cells; IL-6, interleukin 6; MMPs, metalloproteinases; NAFLD, non-alcoholic fatty liver disease; TG, triglycerides; TIMPs, tissue inhibitors of metalloproteinases; TNFα, tumor necrosis factor alpha.

Leptin

Leptin is mainly produced by matured adipocytes, and it is secreted proportionally to the amount of white adipose mass. Different studies have demonstrated the importance of leptin in NAFLD. Knock-out mice for the *ob* gene (*ob/ob*), which codes for leptin, exhibit hepatic steatosis and NASH together with IR, obesity and diabetes [26]. In animal models, it has been demonstrated that leptin may protect from hepatic steatosis at the initial stages of the disease inhibiting gluconeogenesis, lipogenesis, and stimulating β-oxidation in liver. However, with the disease progression, it may act as an inflammatory and fibrogenic factor [29]. Leptin is also secreted by activated HSCs and, through an autocrine effect, promotes cells perpetuation and the synthesis of the α1(I) and α2(I) fibrils, major components of dense and fibrotic ECM [30]. In parallel, in the liver, leptin stimulates the production of TIMP-1 and represses the expression of MMP-1 [26], [31] (Figure 2). Accordingly, the increase in leptin expression in NAFLD would favor hepatic inflammation and fibrosis.

Adiponectin

Adiponectin is an anti-inflammatory adipocytokine, which increases free fatty acid (FFA) oxidation and decreases gluconeogenesis in the liver, improving insulin sensitivity. Therefore, adiponectin presents anti-steatotic effects on the hepatocytes, protecting liver from steatosis. However, opposite to other adipokines, adiponectin is paradoxically decreased in obesity. Therefore, it has been demonstrated that adiponectin serum levels are reduced and inversely associated with the degree of hepatic steatosis, necroinflammation and fibrosis in NAFLD [32], [33]. Furthermore, adiponectin exerts anti-fibrotic action reducing HSC activation and proliferation and inducing apoptosis.

Regarding the possible adiponectin influence on hepatic MMPs, in vitro studies reported that adiponectin would increase MMP-9 activity in primary human hepatocytes [22]. Moreover, Saxena et al. demonstrated that in liver adiponectin downregulates TGF-β, connective tissue growth factor and collagen, and favors matrix degradation by changing the molecular ratio MMP-1/TIMP-1, explaining its anti-fibrotic action [30] (Figure 2). Otherwise, in patients with combined hyperlipidemia, it was reported that adiponectin decreases MMP-2, MMP-9, TIMP-1 and TIMP-2 plasma levels [34]. In accordance, we previously reported an inverse association between plasma adiponectin levels and circulating MMP-2 activity in patients with MS [14] as well as in NAFLD patients, independently of obesity markers. In patients with NAFLD we also observed a negative correlation between MMP-2 plasma activity and circulating adiponectin levels (Table 1), suggesting a positive regulatory role of this adipocytokine on MMPs behavior.

Expert opinion

Different studies have shown that MMPs expression and activity are altered in insulin resistant states. Several factors such as adipokines are involved in the regulation of these enzymes and their inhibitors. It must be taken into account that there are different MMP expression patterns, thus the response to IR is variable in the diverse tissues. This feature is partially responsible for the contradictory results reported until now. In the liver MMPs exert a pro-fibrotic effect under various pathological conditions, such as NAFLD. However, it depends on the stage of the disease and the consequent impact of different regulators on these enzymes and their inhibitors. The consideration of MMPs as a therapeutic target for liver disease still requires further studies of their behavior in patients with different steatosis degrees.

Outlook

The epidemic of obesity and IR in the world during the last decades has been responsible for the incredible increase in the incidence and prevalence of NAFLD. Moreover, as a consequence of the lack of therapeutic treatment a significant progression towards NASH and fibrosis is reported. Different drugs used in the management of IR, such as metformin and thiazolidinediones, have shown an inhibitory effect on MMPs in vitro; however, studies in humans have reported controversial results. It could be due to the unspecific effect of these drugs on the different MMPs and the time of inhibition. The principal challenge of much research nowadays is the design of new specific drugs against different therapeutic targets, including MMPs and TIMPs, which could be used since the beginning of the pathology to prevent NAFLD progression.

Highlights

- Variation in MMPs expression is associated with the fibrotic stage of liver diseases.
- The behavior of MMPs in liver fibrosis process is controversial.
- Patients with NAFLD present higher MMP-2 plasma activity than controls.
- Patients with severe fibrosis present increased MMP-2 plasma activity and decreased MMP-2 liver activity.
- Different adipokines, such as adiponectin would be responsible for MMPs behavior.

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Author Statement

Conflict of interest: The authors declare no conflict of interest.

Informed consent: Informed consent is not applicable.

Ethical approval: The conducted research is not related to either human or animals use.

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