Reactive Oxygen Species and Oxidative Stress in the Pathogenesis of MAFLD
Clare, Kathleen; Dillon, John F.; Brennan, Paul N.

DOI:
10.14218/JCTH.2022.00067

Publication date:
2022

Licence:
CC BY-NC

Document Version
Publisher's PDF, also known as Version of record

Link to publication in Discovery Research Portal

Citation for published version (APA):
https://doi.org/10.14218/JCTH.2022.00067

General rights
Copyright and moral rights for the publications made accessible in Discovery Research Portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from Discovery Research Portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain.
- You may freely distribute the URL identifying the publication in the public portal.

Take down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Download date: 12. Jul. 2022
Abstract

The pathogenesis of metabolic-associated fatty liver disease (MAFLD) is complex and thought to be dependent on multiple parallel hits on a background of genetic susceptibility. The evidence suggests that MAFLD progression is a dynamic two-way process relating to repetitive bouts of metabolic stress and inflammation interspersed with endogenous anti-inflammatory reparative responses. In MAFLD, excessive hepatic lipid accumulation causes the production of lipotoxins that induce mitochondrial dysfunction, endoplasmic reticulum stress, and over production of reactive oxygen species (ROS). Models of MAFLD show marked disruption of mitochondrial function and reduced oxidative capacitance with impact on cellular processes including mitochondrial biogenesis. In excess, ROS modify insulin and innate immune signaling and alter the expression and activity of essential enzymes involved in lipid homeostasis. ROS can also cause direct damage to intracellular structures causing hepatocyte injury and death. In select cases, the use of anti-oxidants and ROS scavengers have been shown to diminish the pro-apoptotic effects of fatty acids. Given this link, endogenous anti-oxidant pathways have been a target of interest, with Nrf2 activation showing a reduction in oxidative stress and inflammation in models of MAFLD. Thyroid hormone receptor β (THRβ) agonists and nuclear peroxisome proliferation-activated receptor (PPAR) family have also gained interest in reducing hepatic lipotoxicity and restoring hepatic function in models of MAFLD. Unfortunately, the true interplay between the clinical and molecular components of MAFLD progression remain only partly understood. Most recently, multimics-based strategies are being adopted for hypothesis-free analysis of the molecular changes in MAFLD. Transcriptome profiling maps the unique genotype-phenotype associations in MAFLD and with various single-cell transcriptome-based projects underway, there is hope of novel physiological insights to MAFLD progression and uncover therapeutic targets.

Citation of this article: Clare K, Dillon JF, Brennan PN. Reactive Oxygen Species and Oxidative Stress in the Pathogenesis of MAFLD. J Clin Transl Hepatol 2022. doi: 10.14218/JCTH.2022.00067.

Introduction

Metabolic-associated fatty liver disease (MAFLD) is now the most common chronic liver condition worldwide and will soon be the leading indication for liver transplantation. MAFLD represents a pathological spectrum of liver injury ranging from simple steatosis to liver fibrosis with an evolutionary course to cirrhosis. Approximately 30% of adults in the general population have MAFLD, 10–20% have steatohepatitis and in these patients, 20–30% go on to develop cirrhosis within 20 years. The link between MAFLD development and obesity, insulin resistance and type 2 diabetes mellitus is well established with MAFLD considered a hepatic manifestation of the metabolic syndrome. Given the increasing prevalence of these related conditions, the incidence of MAFLD is projected to increase with data suggesting a 56% rise over the next decade. Although MAFLD is typically associated with a western lifestyle, data demonstrates a rapid increase in disease burden in developing counties. The MAFLD disease continuum has the associated sequelae of end-stage liver disease and hepatocellular carcinoma (HCC). Patients with significant fibrosis have a higher risk of detrimental outcomes compared to those with simple steatosis and alarmingly, these patients may develop HCC without progression to cirrhosis first. The MAFLD disease continuum has the associated sequelae of end-stage liver disease and hepatocellular carcinoma (HCC). Patients with significant fibrosis have a higher risk of detrimental outcomes compared to those with simple steatosis and alarmingly, these patients may develop HCC without progression to cirrhosis first.

MAFLD pathogenesis

The pathogenesis of MAFLD is complex and still not fully understood; it remains a challenge to stratify and identify specific drug targets and currently there are no licensed therapies for its management. The original two hit hypothesis proposed by Day et al. is now largely obsolete having been replaced with the multiple parallel hits hypothesis.
The development of MAFLD is dependent on multiple cumulative insults including a nonmodifiable genetic susceptibility. MAFLD has been shown to be polygenic with the PNPLA3, MBOAT7 and TM6SF2 gene variants identified as predisposing risk for disease development. Additional factors which have a putative pathogenic role are excessive dietary intake, products of the microbiota and/or maladaptation to environmental stimuli. The multiple, parallel hits model suggests that in the presence of significant lipid accumulation in hepatocytes and systemic and hepatic insulin resistance, there are multiple coincident metabolic alterations which leads to an imbalance between free radical production from gut and adipose tissue and anti-lipotoxic protective mechanisms of the liver. The pathophysiological mechanisms involved in this include endoplasmic reticulum (ER) stress, excessive generation of reactive oxygen species (ROS) and diminished catabolism of fatty acids which lead to a pro-inflammatory state. The mechanisms, which have associations with insulin resistance, involve numerous cell responses, pro-inflammatory cytokines, chemokines, and toll-like receptors with complex interaction profiles. It is imperative to note that although ROS are agents of damage, one of their most important biological roles is cell signaling through acting as sensors of cellular stress and setting the oxidative tone of the cell.

Overall, the evidence suggests that MAFLD progression is a dynamic two-way process relating to repetitive bouts of metabolic stress and inflammation interspersed with endogenous anti-inflammatory reparative responses. An overlay of these pathophysiological concepts underlying MAFLD are best illustrated by means of the schematic in Figure 1. Given the current lack of treatment for MAFLD, the soaring global epidemic presents a major challenge. It is becoming increasingly essential to uncover the specific pathogenic mechanisms underlying this disease in order to identify pharmacological targets for novel therapies.

### Role of ROS in MAFLD progression

One of the most important roles of ROS is cellular signaling. Through modulating transcription factors, ROS have a key role in cell proliferation and differentiation, metabolism, and immune defense mechanisms. ROS are continually produced by various intracellular organelles including mitochondria, ER, and peroxisomes as by-products of normal cellular metabolism. In normal physiology, ROS are buffered at a steady state in order to maximize cellular redox signaling. Oxidative stress describes an imbalance between the production of reactive oxygen species (ROS) and the host antioxidant scavenging capacity in favor of the former. Oxidative stress and ROS are intrinsically linked in the pathogenesis of MAFLD. The discrepancy between ROS generation as a potent proinflammatory, and antioxidant defense proponents; potentiates both DNA and cellular injury. The proinflammatory cascades may be propagated through increasing pro-oxidant signaling, or relative antioxidant dysfunction and there likely exists an important...
inflection point beyond which fibrosis develops. ROS signaling is therefore considered as a strong pro-determinant in hepatic fibrogenesis. These complex pathways likely involve a number of coexistent pro-oxidative triggers which synergistically interact in concert with mitochondrial dysfunction as a principle potentiator of OS.

Mitochondrial dysfunction in MAFLD

Mitochondria are complex organelles with diverse roles in energy metabolism, cell signaling, and calcium homeostasis. Mitochondria are central to cellular energy modulation, given their inherent role in ATP regulation. There are intrinsic gate-keeper mechanisms responsible for maintaining mitochondrial integrity; known as the mitochondrial quality control (MQC) system. This MQC comprises facets including mitochondrial fission and fusion, mitophagy and redox regulation. Excessive and dysregulated ROS production within the mitochondrial matrix may damage constituent structures including mitochondrial membrane, mitochondrial DNA (mtDNA) and may induce pro-apoptotic pathways including mitochondrial autophagy, a process also known as mitophagy. These interactions are illustrated in Figure 2.

Structurally, mitochondria are composed of an inner mitochondrial membrane, and an outer mitochondrial membrane that are separated by an intermembranous space. Mitochondria contain double-stranded circular DNA (mtDNA) that encodes 13 polypeptides of the respiratory chain complexes, including adenosine triphosphate (ATP) synthase and additional RNAs responsible for intramitochondrial translation.

Functionally, mitochondrial energy coupling occurs via the electron transfer chain (ETC), whereby ATP is generated by controlled movement of electrons along the ETC from a high energy state to low energy state in a step wise fashion. This process is facilitated by sequential reduction of nicotinamide adenine dinucleotide and flavin adenine dinucleotide to NADH and FADH2 respectively, which subsequently are donated at regulatory points in the ETC. The energy potential released from this electron transfer potentiates a proton gradient and facilitates the phosphorylation of ADP to ATP in a process called oxidative phosphorylation (OXPHOS). Given the fundamental role of the mitochondria in energy regulation, mitochondrial dysfunction appears to be a key component in the pathogenesis of MAFLD.

There are a number of essential nuclear-encoded proteins within mitochondria that have specific functions, but together play a role in mitochondrial biogenesis, the formation of new mitochondria. Peroxisome proliferation-activated receptor gamma (PPARγ) is a ligand-activated transcription factor with a spectrum of function including regulation of mitochondrial production and function, redox balance, and fatty acid oxidation. PPARγs coactivator-1 alpha (PGC-
Role of lipid dysregulation in MAFLD pathogenesis

The most direct etiology of MAFLD is disequilibrium of lipid metabolism, and hepatic steatosis is the result of an imbalance in lipid production and degradation. The link between diet and MAFLD is well established. Excessive consumption of refined carbohydrates, saturated fats, and animal protein is associated with the development of steatosis and MAFLD progression. Obesity results from a failure to regulate body mass and is the result of excess energy intake, reduced energy expenditure, or both. Obesity causes a marked increase in visceral adipose tissue and profound changes to its function, which has significant secondary effects on the liver. In obese individuals, visceral adipose tissue is highly biologically active. Adipocytes develop an inflammatory phenotype, become necrotic, and secrete cytokines into systemic circulation, which modulates hepatic immune function and induces hepatocyte death. Triglyceride storage in adipocytes is also disrupted, this leads to the inappropriate delivery of free fatty acids (FFAs) to nonadipose tissue causing lipotoxicity, a crucial event in MAFLD pathogenesis. Delivery of FFAs to the liver impairs insulin sensitivity, causes transcription of sterol responsive element binding protein 1c (SREBP-1c) and promotes de novo lipogenesis which further contributes to steatosis.

In MAFLD, as hepatic lipid deposition increases, intracellular processes become overwhelmed, and ROS generating mechanisms are potentiated. In general, there are three primary sources of FFAs, which precipitate hepatic lipid accumulation. They are peripheral lipolysis, de novo lipogenesis and dietary. Hepatic FFAs typically have two major metabolic fates, they undergo mitochondrial beta oxidation or they undergo esterification to form triglycerides. In MAFLD, the increased influx of FFAs overwhelms hepatic metabolic capacity, causing failure of beta oxidation and mitochondrial dysfunction. The surplus of fatty acids can, instead, be converted into triglycerides, stored as lipid droplets, and partially released into circulation as very low-density lipoproteins. The excess FFAs can also act as a substrate for the generation of lipotoxic lipid species such as ceramides and diacylglycerols. These lipotoxins are known to cause hepatocellular stress and, in combination with the free pool of hepatic fatty acids, induce mitochondrial dysfunction and endoplasmic reticular stress. They also activate NADPH oxidase (NOX). An enzyme complex that catalyzes the production of superoxide free radicals, a major source of cellular ROS. These three primary mechanisms account for the increased production of ROS from hepatic lipid deposition, O$_2^-$, H$_2$O$_2$, malondialdehyde (MDA) and 4-hydroxy-2-nonenal (4-HNE) in particular. The elevated ROS signals modify insulin and innate immune signaling, and alter the expression and activity of essential enzymes involved in lipid hemostasis. In combination, these effects result in the redox-dependent dysregulation of hepatic lipid metabolism seen in MAFLD.

One further, more recently identified, endogenous influence on hepatic lipid regulation is thyroid hormone (TH) by its activation of THβ receptor in the liver. TH has been shown to have an essential role in hepatic cholesterol synthesis and fatty acid metabolism. Through its ability to alter the function of transcription factors, moderate cell signaling cascades, and through binding to proteins other than TH receptors, TH can modulate gene expression for hepatic fatty acid biosynthesis, cholesterol, and metabolism. The ability of TRβ1 selective agonism of TH in modulating lipid homeostasis may be partly explained by increased clearance, and increased hepatic Ldlr expression in addition to synergistic effects on cholesterol 7α-hydroylase (CYP7A1) related cholesterol synthesis. Various studies have demonstrated the relationship between TH levels and MAFLD, with patients who have hypothyroidism and high levels of TSH.
with a negative mutation in THβ, hepatic steatosis was pre-correlating with more extensive steatosis. In mouse models at an increased risk of MAFLD, with greater TSH elevation and decreased THR-mediated fatty acid β-oxidation.

Models was thought to be caused by increased PPARγ signaling by 4–5 months of age. Hepatic lipid accumulation in the models is consistent evidence of decreased Nrf2, Tfam, and PGC-1 expression in models of MAFLD.17,40

ROS have been shown to activate NLRP3 and lead to down-regulation of cytoprotective enzymes and proteins, there is a reduction in ROS generation with subsequent reduction in oxidative damage, inflammation and cell apoptosis.42 Research has shown Nrf2 is a key modulator in the natural defense against MAFLD and studies to support this show that loss of Nrf2, or Nrf2 deletion accelerates the progression of MAFLD in mouse models.43,44

Given these findings, there is enormous interest in exploiting the therapeutic potential of Nrf2 activation. Du et al.45 found that though using osteocalcin, a small protein found in bone and dentin which activates Nrf2, there could be reduction in oxidative stress and inhibition of the JNK pathway which plays an essential role in MAFLD pathogenesis and thus improving disease progression. Studies have shown that ezetimibe (a Niemann-Pick-C1-Like 1 inhibitor used to treat hypercholesterolemia) and green tea extract both promote the protective features of Nrf2 against hepatic lipid deposition and the inflammatory response in MAFLD. Despite this, Nrf2-related therapeutics remain unlicensed for use in MAFLD, and the development of promising agents to target oxidative stress in management of this disease remains challenging.46,47

Intracellular hepatic lipid accumulation can activate Kupffer cells and the release of pro-inflammatory cytokines including IL1, TNF-α and IL6 whose effects are known to enhance MAFLD progression to fibrosis and cirrhosis.48 A study in mice with diet-induced obesity showed that pharmacological blockade of IL1 using anakinra, a recombinant IL1 receptor antagonist, significantly improved hepatic steatosis by decreasing inflammation and lipogenic gene expression.49 Although there is currently a lack of research in its effects in humans with MAFLD, studies of anakinra in patients with type 1 and 2 diabetes showed an improvement in insulin sensitivity and a reduction is systemic inflammation, and thus could be a promising therapeutic target in the future for MAFLD.50,51

Another attractive therapeutic target, given the relationship between thyroid hormone, hepatic fatty acid, cholesterol metabolism and MAFLD, is the THRβ receptor. There are currently a number of THRβ agonists demonstrated the potential to reduce hepatic lipotoxicity and restore function in models of MAFLD in clinical trials. Resmetirom is a highly specific THRβ agonist designed to improve MAFLD. Its selectivity to the THRβ receptor enhances its safety profile, as it has therapeutic effectiveness without the unwanted systemic events in the heart, bones, and thyroid axis through activation of THRβ receptors. One randomized, double-blind, placebo-controlled trial in patients with biopsy-confirmed MAFLD showed that treatment with resmetirom resulted in a significant reduction in hepatic fat at both 12 and 36 weeks. Those positive results have initiated a phase 3 multinational
Lastly, the PPAR family of nuclear receptor transcription factors, has gained interest as a novel therapeutic target in MAFLD as their dysregulation is known to affect lipid metabolism, contribute to insulin resistance, inflammation, and hepatic fibrogenesis. There are three PPAR isoforms, PPARα, PPARβ, and PPARγ, which have varied expression among cell types and tissues. PPARα is the main form expressed in the liver, but all three isoforms have a role in the regulation of normal liver function. Multiple studies have looked into the benefits of activating one or several PPAR forms in preclinical models of liver disease with positive outcomes. However, pharmacological activation of all three PPAR isoforms concomitantly has only recently been investigated. A number of studies have shown that pioglitazone, a selective PPARγ agonist, significantly improves hepatic steatosis, inflammation and insulin resistance, independent of blood glucose control, in patients with type 2 diabetes. Lanibranor is a pan-PPAR agonist that has been shown to reduce portal hypertension and hepatic fibrosis in preclinical models of decompensated cirrhosis in both cirrhotic rat models and human liver cells from patients with cirrhosis. Although pioglitazone remains the only PPAR agonist with a proven protective role in human MAFLD, these promising findings support further work in the development of PPAR agonists for their use in liver disease. An overview of potential therapeutic targets and agents in the future of MAFLD is outlined in Table 1.

Future directions

With the rapidly rising global prevalence of MAFLD and its associated healthcare costs, there is increasing focus on the development of novel therapies to prevent, manage or even cure this disease. The pathogenesis of MAFLD is complex and driven by dynamic molecular mechanisms with multifaceted, parallel signaling pathways as shown in Figure 1. Unfortunately, the direct interplay between the clinical and molecular components linked to MAFLD progression remain only partly understood. To date, potential treatments of MAFLD have typically targeted one of the hallmark pathophysiological risk factors driving the disease, inflammation, steatosis, fibrosis, or the gut microbiota. However, given its heterogeneous nature, managing MAFLD through alteration of one mechanism is nearly impossible. A general overview of the implicit pathophysiological mechanisms linking ROS to MAFLD development is presented in Figure 3.

In order to gain a better understanding of the complex biological processes underlying MAFLD development, multiomics-based strategies have been adopted by researchers for hypothesis-free analysis of the molecular changes in MAFLD. The underlying genomic structure of every cell
Clare K. et al: Development of MAFLD as influenced by ROS and OS

within a given organism is principally the same. However, the physiological fate of a cell depends on the intrinsic cellular genome expression signature. Differing phenotypes are the result of genotypic alterations and their varying patterns of expression, abnormalities of this typically result in disease. Transcriptome profiling is required for the mapping of this unique genotype-phenotype association, and there are a number of single-cell transcriptome-based projects underway that should provide novel physiological insights from which translational targets will be derived.64

Funding

None to declare.

Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Basic source acquisition and analysis (KC, JFD, PNB), drafting of the manuscript, and manuscript taking. Preparation of the document (KC), and original research design and providing input in critical manuscript writing and editing (JFD, PNB). All authors approved the final draft of manuscript. PNB is the guarantor of this article.

References

Journal of Clinical and Translational Hepatology 2022;8:8

Clare K. et al.: Development of MAFLD as influenced by ROS and OS


