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Role of gut microbiota and oxidative stress in the progression of non-alcoholic fatty liver disease to hepatocarcinoma: Current and innovative therapeutic approaches

Antonella Borrelli[a,*], Patrizia Bonelli[a], Franca Maria Tuccillo[a], Ira D. Goldfine[b], Joseph L. Evans[c], Franco Maria Buonaguro[a], Aldo Mancini[d]

[a] Molecular Biology and Viral Oncology Unit, Istituto Nazionale Tumori IRCCS "Fondazione G Pascale", 80131 Napoli, Italy
[b] University of California, San Francisco, CA, USA
[c] P & N Development Venture, Saint Louis, MO 63103, USA
[d] Leadhexa Biotechnologies Inc., Belvedere, CA, USA

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ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) represents the most common chronic liver disease in industrialized countries. NAFLD progresses through the inflammatory phase of non-alcoholic steatohepatitis (NASH) to fibrosis and cirrhosis, with some cases developing liver failure or hepatocellular carcinoma (HCC). Liver biopsy remains the gold standard approach to a definitive diagnosis of NAFLD and the distinction between simple steatosis and NASH. The pathogenesis of NASH is still not clear. Several theories have been proposed ranging from the "Two Hit Theory" to the "Multiple Hit Theory". However, the general consensus is that the gut microbiota, oxidative stress, and mitochondrial damage play key roles in the pathogenesis of NASH. The interaction between the gut epithelia and some commensal bacteria induces the rapid generation of reactive oxygen species (ROS). The main goal of any therapy addressing NASH is to reverse or prevent progression to liver fibrosis/cirrhosis. This problem represents the first "Achilles' heel" of the new molecules being evaluated in most ongoing clinical trials. The second is the inability of these molecules to reach the mitochondria, the primary sites of energy production and ROS generation. Recently, a variety of non-pharmacological and pharmacological treatment approaches for NASH have been evaluated including vitamin E, the thiazolidinediones, and novel molecules related to NASH pathogenesis (including obeticholic acid and elafibranor). Recently, a new isoform of human manganese superoxide dismutase (MnSOD) was isolated and obtained in a synthetic recombinant form designated rMnSOD. This protein has been shown to be a powerful antioxidant capable of mediating ROS dismutation, penetrating biological barriers via its uncleaved leader peptide, and reducing portal hypertension and fibrosis in rats affected by liver cirrhosis. Based on these distinctive characteristics, it can be hypothesized that this novel recombinant

Abbreviations: ATP, Adenosine 5c-triphosphate; ALT, Alanine aminotransferase; aa, Amino acid; ASK1, Apoptosis signal- regulating kinase 1; AST, Aspartate aminotransferase; BASH, Both alcoholic and non-alcoholic liver disease; CCR2/CCR5, C-C chemokine receptor types 2 and 5; CVC, Cenicriviroc; CASH, Chemotherapy-associated steatohepatitis; JNK, c-Jun N-terminal kinase; CD14, Cluster of differentiation 14; PGC-1α, Coactivator peroxisome proliferator-activated receptor-γ-1α; CS + WR, Cold storage and warm reperfusion; Cu/ZnSOD, Copper/zinc superoxide dismutase; DPP-4 inhibitor, Dipeptidyl peptidase 4 inhibitor; DASH, Drug-associated steatohepatitis; ER, Estrogen receptor; ecSOD, Extracellular Cu/ZnSOD; FXR, Farnesoid X receptor; FIAF, Fasting-induced adipose factor; FDA, Food and drug administration; FGF19, Fibroblast growth factor 19; FGF21, Fibroblast growth factor 21; FADH, Flavin adenine dinucleotide; FFA, Free fatty acids; γgt, Gamma-glutamyl transferase; GF, Germ-free; GIT, Gastrointestinal tract; GLP-1, Glucagon-like peptide-1; *H. pilory*, *Helicobacter pylori*; HSCs, Hepatic stellate cells; HVPG, Hepatic venous pressure gradient; HCC, Hepatocellular carcinoma; •OH, Hydroxyl free radicals; IL-10, Interleukin-10; IL-6, Interleukin-6; LPS, Lipopolysaccharide; LPB, Lipopolysaccharide-binding protein; LPL, Lipoprotein lipase; NN2211, Liraglutide; LOXL, Lysyl oxidase and lysyl oxidase-like; MDA, Malondialdehyde; MnSOD, Manganese superoxide dismutase; Mkt, Market; MS, Metabolic syndrome; H_2, Molecular hydrogen; O_2, Molecular oxygen; RG-125 AZD4076, N-acetylgalactosamine (GalNAc)-conjugated anti-miR-103/107 oligonucleotide; NAP, *H. pylori*-induced neutrophil-activating protein; NAS, NAFLD activity score; NKT, Natural killer T; NADH, Nicotinamide adenine dinucleotide; NO, Nitric oxide; NAFLD, Non-alcoholic fatty liver disease; NASH, Non-alcoholic steatohepatitis; NF-κB, Nuclear factor kappa; INT-747, Obeticholic acid (OCA); PNPLA3, Patatin-like phospholipase 3; PAMPs, Pathogen-associated molecular patterns (PAMPs); PPAR, Peroxisome proliferator-activated receptor; PIVENS, Pioglitazone versus Vitamin E versus Placebo; PASH, PNPLA3-associated steatohepatitis; HPC, Primary hepatic carcinoma; ROS, Reactive oxygen species; ETC, Respiratory electron transport chain; Saroglitazar-ZYH1, [(*S*)-α-ethoxy-4-{2-[2-methyl-5-(4-methylthio) phenyl)]-1H-pyrrol-1-yl]-ethoxy})-benzenepropanoic acid magnesium salt]; SSAO, Semicarbazide-sensitive amine oxidase; PXS-4728A, SSAO/VAP-1 inhibitor BI 1467335; SCD1, Stearoyl-coenzyme A desaturase 1; O_2^-, Superoxide anion; SOD, Superoxide dismutase; TZDs, Thiazolidinediones; TLR, Toll-like receptor; TNF, Tumor necrosis factor; VAP-1, Vascular adhesion protein-1; VLX103, Venlafaxine-103

* Corresponding author.
E-mail address: a.borrelli@istitutotumori.na.it (A. Borrelli).

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protein (rMnSOD) potentially represents a new and highly efficient adjuvant therapy to counteract the progression from NASH to HCC.

1. Introduction

Fatty liver is an emerging medical problem. This condition is often discovered at the same time during ultrasound examinations performed for other purposes. Although the possible etiological factors have been investigated, in a high proportion of cases, the cause of the disease remains unidentified. These cases, defined as “metabolic syndrome” are often related to other diseases, or to other predictive parameters (hyperglycemia, dyslipidemia, high blood pressure, and abdominal obesity) and are classified as non-alcoholic fatty liver disease (NAFLD). This condition is one of the most common benign liver disorders in modern societies, and represents the first stage of a process that may evolve into an inflammatory phase defined as non-alcoholic steatohepatitis (NASH). Subsequently, this process can lead to fibrosis, cirrhosis, and even hepatocellular carcinoma (HCC) with high rates of morbidity and mortality [1]. A schematic diagram of these processes is shown in Fig. 1. Over the years, several terms have been used to describe NASH, such as pseudo-alcoholic hepatitis, alcohol-like hepatitis, fatty liver hepatitis, steatonecrosis and diabetic hepatitis. More recently, abbreviations such as BASH (both alcoholic and non-alcoholic liver disease), DASH (drug-associated steatohepatitis), CASH (chemotherapy-associated steatohepatitis), and PASH (PNPLA3-associated steatohepatitis) have been adopted to distinguish the various etiologies [2]. In this review, we provide an updated overview of NAFLD/NASH and progression to cirrhosis/HCC, with a particular focus on the roles of the gut microbiota and oxidative stress, as well as the prospects for novel therapies.

2. Worldwide incidence of NAFLD

The prevalence of NAFLD in the general population is approximately 6–35%, with a median incidence of 20% [2,3]. Worldwide distribution, referred to a meta-analysis carried out with studies conducted between the years 2000–2015, is highly variable as shown in Table 1 [4,5].

In the general population of Western countries, the incidence of NAFLD is 20–30%, with a prevalence of approximately 30% among adults in the United States (US) and 25% in Italy [6].

Patients with NAFLD have higher overall mortality than control patients [1], and most deaths are due to cardiovascular events [7]. Male sex, older age, increased waist circumference, low high-density lipoprotein levels, and the presence of metabolic syndrome are the independent predictors of mortality in patients with NAFLD [8]. In healthy men, increased serum alanine aminotransferase (ALT) levels, even within the reference range, are an independent predictor of NAFLD [9], although this parameter is not predictive of death. A reversal of an ALT/AST (aspartate aminotransferase) ratio of more than 1 is an index of advanced fibrosis [1]. Other predictors of NAFLD are insulin resistance (which can induce dyslipidemia and atherosclerosis), endothelial dysfunction, alteration of the left ventricular energy metabolism, stroke, and increased expression of inflammation mediators [10]. NAFLD is present in 80–90% of obese individuals, in 30–50% of diabetics and approximately 90% of patients with hyperlipidemia [6].

3. Worldwide incidence of NASH

Among the general population, the worldwide incidence of NASH is 5–7%, as reported in a study performed by Pereira K. et al. in 2015 [11], with 30–40% having raised liver enzymes and progressing from a chronic liver disease to cirrhosis and hepatocarcinoma. Among patients with NASH, 34–50% remain stable and 18–29% improve the histological aspect of their liver, while 26–37% progress to fibrosis, with 9–25% of these patients going on to develop cirrhosis [11,12]. NASH is involved in approximately 30–40% of deaths caused by liver disease. In terms of progression to HCC, 0–0.5% of patients progress from simple hepatic steatosis, 0–2.8% from NASH and 40–62% from cirrhosis [11]. The risk factor of NAFLD/NASH is similar in all countries [6,13]. The evolution of NASH to fibrosis/cirrhosis depends critically on the initial stage of inflammation [14]. Cirrhosis is not always present and it has been reported that 41.7% of patients develop HCC without showing cirrhosis [15,16]. Among patients with NASH, 20% progress to cirrhosis over a 20-year period with increased risk of liver failure or HCC resulting in transplantation or death [17]. After hepatitis C virus, NASH has become the leading etiology of liver transplantation due to HCC in the US, with a four-fold increase reported over the period from 2002 to 2012 [18]. However, HCC is not necessarily associated with cirrhosis, but depends on metabolic diseases (diabetes, obesity, and insulin resistance) [19].

4. NAFLD/NASH: pathogenesis

A subset of patients with NAFLD develops NASH; however, the mechanism is poorly understood and the pathogenesis of NASH is unclear although the current scientific consensus accepts the concept of the “Multiple Hit Theory” [20] (Fig. 2) rather than the “Two Hit Theory” [21] (Fig. 3).

Until recently, hepatic steatosis [22] and oxidative stress [23] – the latter also caused by changes in the gut microbiota [24,25] – were seen

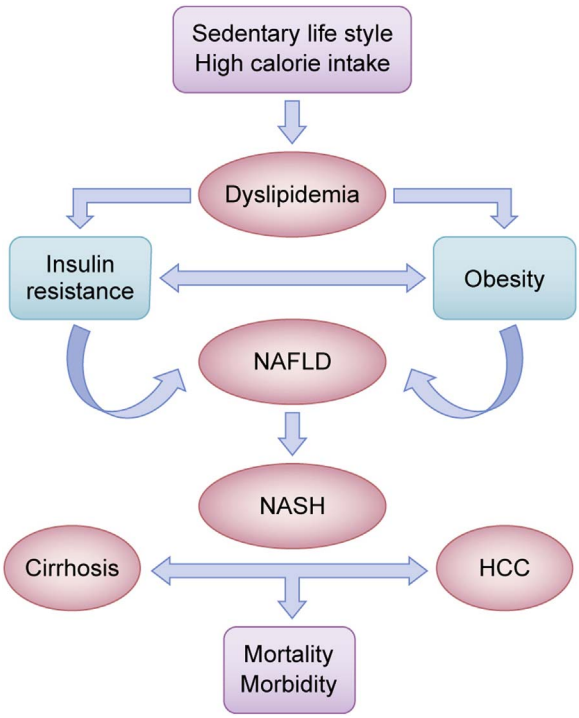

Fig. 1. Schematic representation of progression from NAFLD/NASH to cirrhosis/HCC. The history of non-alcoholic fatty liver disease (NAFLD). This term assembles a wide spectrum of conditions ranging from dyslipidemia to steatohepatitis (NASH). Lifestyle factors, such as sedentary lifestyle and high calorie intake combined with insulin resistance and several others genetic and epigenetic factors induce the progression of NASH to cirrhosis and hepatocarcinoma (HCC) with its clinical consequences.