Opinion: Intestinal Microbiome, Endotoxins, Cytochrome P450 2E1, and the Gut-Liver Axis in Alcoholic Liver Disease

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Abstract

Alcoholic liver disease (ALD) is linked to dozens of mechanistic events with a more recent focus on a contributing causative role of the intestinal tract. Experimental and clinical evidence suggests that endotoxins produced by intestinal bacteria leave the leaky gut whereby the intestinal microsomal ethanol-oxidizing system (MEOS) with CYP 2E1 as its major constituent plays a permissive role. Endotoxins in the systemic circulation enter the liver and are taken up by hepatocytes and Kupffer cells. Here, hepatic MEOS and CYP 2E1 facilitate initiation and aggravation of liver injury causally related to alcohol and acetaldehyde as its first toxic metabolite. Reactive oxygen species (ROS) generated during ethanol metabolism via the hepatic NADPH-dependent MEOS and CYP 2E1 will help establish a gut-liver axis that contributes to the development of alcoholic liver disease in addition to various other mechanistic steps prevailing in hepatocytes and non-parenchymal liver cells. The conclusion is reached that intestinal and hepatic MEOS in association with CYP 2E1 and ROS contribute to emerging alcoholic liver injury through a gut-liver axis, substantiating a close relationship between the gut and the liver.

Keywords: Alcoholic Liver Disease; Cytochrome P450 2E1; Endotoxemia; Intestinal Bacteria; Microsomal Ethanol-Oxidizing System; Reactive Oxygen Species; Gut-Liver Axis

Abbreviations

ADH: Alcohol Dehydrogenase; ALD: Alcoholic Liver Disease; CYP: Cytochrome P450; CYP 2E1: Cytochrome P450 2E1; MEOS: Microsomal Ethanol-Oxidizing System; NAFLD: Nonalcoholic Fatty Liver Disease; NASH: Nonalcoholic Steatohepatitis; ROS: Reactive Oxygen Species

Introduction

Until the middle of the sixties the concept prevailed that alcoholic liver disease (ALD) is exclusively the result of malnutrition observed among patients in poor nutritional state and with a medical history of prolonged alcohol abuse [1]. This initial nutrition-based thesis was then replaced by more stringent clinical and experimental evidence of a direct toxic effect of alcohol and more so of acetaldehyde as its first oxidation product [1-4]. The focus was now on increased hepatic lipogenesis, diminished hepatic release of lipoproteins, increased mobilization of peripheral fat, augmented hepatic uptake of circulating lipids, decreased fatty acid oxidation, increased collagen production, decreased collagen degradation, and various other metabolic disturbances [1,2]. A plethora of studies proposed another dozens of molecular pathogenetic events leading to ALD as provided in the relevant literature [3-10]. A unifying mechanistic approach was missing

[9,10]. Mechanistic concepts included not only the hepatocytes but also various non-parenchymal cell in the liver [1-10]. Within the liver cell, various metabolic disturbances were described with much emphasis placed on pathways of alcohol and acetaldehyde metabolism, especially the hepatic microsomal ethanol-oxidizing system (MEOS) with cytochrome P 450 (CYP) as its major constituent, preferentially the isoenzyme CYP 2E1 [10]. Both hepatic MEOS activity and CYP 2E1 content are upgraded due to chronic alcohol consumption. Conditions are even more complex considering the possible supporting role of the intestinal tract for initiation and perpetuation of ALD [11-14], provoked by the actions of endotoxins derived from intestinal bacteria [14]. In addition, a large body of evidence accumulated that the mucosa of the intestinal tract exhibits MEOS activity and contains CYP 2E1, findings similar to those in the liver.

The present article focuses on the gut-liver axis in ALD, a largely neglected topic in the alcohol research area. Of special interest is the significance of MEOS and CYP 2E1 present in the gut and the liver as well as the role of reactive oxygen species (ROS) initiating and perpetuating alcoholic liver injury.

Literature search and source

The PubMed database was used to identify publications for the following terms: Intestinal microbiome; gut-liver axis; intestinal bacteria; endotoxins; intestinal cytochrome P450, hepatic cytochrome P450, intestinal microsomal ethanol-oxidizing system; hepatic microsomal ethanol-oxidizing system; reactive oxygen species. Limited to English language, publications of the first 50 hits from each searched segment were analyzed for suitability of this review article. The search for additional publications was completed on 24 November 2018. The final compilation consisted of original papers, consensus reports, and review articles. The most relevant publications were included in the reference list of this article.

Hepatic MEOS

MEOS activity was described in the liver of humans [15] and animals [1,15-20]; it adaptively increases in activity following prolonged alcohol abuse [15-22]. This enzyme upregulation enhances alcohol metabolism [22,23] and increases the production of acetaldehyde, considered a highly toxic product responsible for many metabolic and injurious events of the liver [1,17,24]. Various constituents represent MEOS (Figure 1) [8,10].
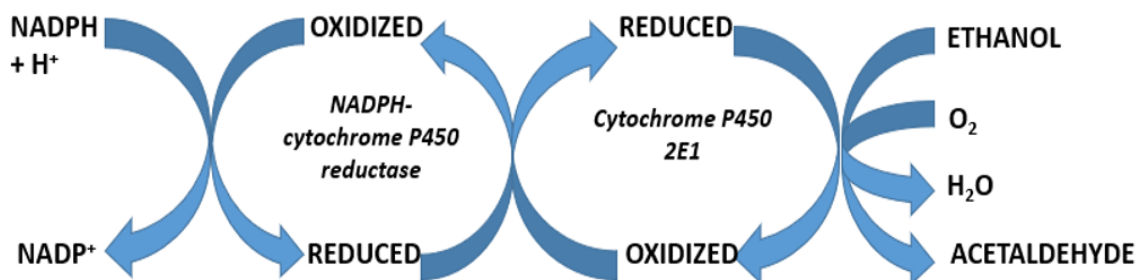
Figure 1: *Constituents of hepatic MEOS. Hepatic microsomal cytochrome P450 2E1 and NADPH-cytochrome P450 reductase are obligatory constituents of the microsomal ethanol-oxidizing system (MEOS), the metabolic reaction requires also phospholipids but the site of their reaction is unknown [8,10]. Reproduced from a previous report [8], with permission of the Publisher Taylor & Francis (Didcot, UK).*

Hepatic CYP 2E 1

Microsomal CYP 2E1 was isolated from human liver, purified, and described in detail [25]. Increased hepatic levels of CYP 2E1 are common features in alcoholic patients [26,27], findings supported by animal studies that allow transfer of experimental CYP 2E1 data to humans [28]. It was also reported that CYP 2E1 may be present in the mitochondria of hepatocytes [29-34], a topic that merits further discussion regarding mitochondrial ethanol metabolism and initiation of liver injury [10,35].

Intestinal MEOS

The gastrointestinal tract of animals exhibits MEOS activity that is induced following chronic alcohol consumption but its quantitative role in overall alcohol metabolism has not yet been studied [36-38]. More specifically, experimental studies indicated the presence of MEOS activity in the stomach [37], small intestine [36,37], colon [37,38], as well as rectum [37]. The presence of MEOS in various segments of the gastrointestinal tract suggests that NADPH-cytochrome P450 reductase and phospholipids are also present in the gastrointestinal mucosa, otherwise MEOS would not function, an assumption based on studies in the liver whereby the reductase, phospholipids, and CYP are essential components of hepatic MEOS [39-42]. Alcohol dehydrogenase (ADH), another enzyme capable of oxidizing ethanol to acetaldehyde, may be found in the gastrointestinal tract [37]. The reaction via ADH produces reducing equivalents in form of NADH + H^+ [1,10], whereas alcohol oxidation via MEOS requires reducing equivalents as NADPH + H^+ [1]. If MEOS and ADH are present in the same mucosal cell, they may exert joint actions through exchange of reducing equivalents and speed up the metabolism of ethanol to its highly toxic acetaldehyde in a way similar to the liver cell [10]. Via intestinal CYP 2E1, the intestinal MEOS modifies microbiome conditions and endotoxin generation [10,43], an important aspect not considered in some reports [11-13].

Intestinal CYP 2E1

In line with the presence of MEOS in the gastrointestinal tract of animals [36-38], there is co-existence with CYP 2E1 in the small intestine and colon [44]. Additional immunochemical studies on the localization of the ethanol-inducible CYP 2E1 in the rat alimentary revealed the occurrence of immunoreactive CYP 2E1 only in the duodenal and jejunal villous cells but not in the ileum or distal colon of animals receiving the control diet [45]. After ethanol treatment, however, CYP 2E1 was now expressed also in the proximal colon, associated with an increased CYP 2E1 content in the duodenum and jejunum as compared to animals receiving the control diet [45].

What is even more, CYP 2E1 is found in the colon of humans, with higher mRNA levels of CYP 2E1 in the descending colon and the sigmoid colon as compared to the ascending colon [46]. For patients with an alcohol problem, no respective data on CYP 2E1 in the gastrointestinal tract are available but an upregulation can be assumed. Resulting from incomplete reaction steps during intracellular oxygen split, CYP generates reactive oxygen species (ROS) [8,10]. Some ROS will be needed for the function of MEOS [10]. Intestinal CYP 2E1 is part of the intestinal microbiome and may contribute to the initiation of ALD [10,43].

Reactive oxygen species

Among the most important ROS, generated by MEOS, CYP 2E1 and ethanol, are the intermediates like hydroxyethyl radical, ethoxy radical, acetyl radical, singlet radical, hydroxyl radical, alkoxyl radical, and peroxyl radical; reactions leading to ROS also require molecular oxygen and NADPH + H^+ as electron donor [10]. These types of microsomal ROS constitute the term of hepatic microsomal oxidative stress [10,35], whereas CYP 2E1 if found in the hepatic mitochondria may cause hepatic mitochondrial oxidative stress [10,31-35]. In addition to this hepatic ROS, intestinal ROS is produced by CYP 2E1 in cells of the intestinal tract [36-38,44-46] and cause intestinal oxidative stress. Apart from alcoholic liver injury, various other conditions exist, for which ROS is considered as a causative factor. Among these are cancer [47] and ROS caused specifically by CYP 2E1 triggers nonalcoholic fatty liver disease (NAFLD) or nonalcoholic steatohepatitis (NASH) commonly associated with overweight or obesity [10,48-52].

The increased generation of ROS following prolonged alcohol abuse and upgrading of CYP 2E1 has thoroughly been investigated [19,20,53-58]. Reactive intermediates are responsible for ethanol oxidation in hepatic microsomes and likely mitochondria [10,19,20,53-55]. Regarding MEOS activity and its association with lipid peroxides, there is uncertainty about the possible mechanistic role of phospholipids as integral constituents of the native microsomal membranes harboring MEOS [10]. In analogy to many drugs and chemicals,

ethanol functions as a substrate for microsomal CYP through an oxidation process, which may partially be incomplete and thereby produce reactive oxygen forms [10]. Substrates such as ethanol bind to the ferric (3$^+$) iron of the cytochrome P450 as the initial metabolic step. This oxidation reaction involves the cytochrome P450 cycle and is complex due to electrons provided by NADPH + H$^+$ and the introduction of oxygen that is split and generates thereby various types of ROS (Figure 2) [59].
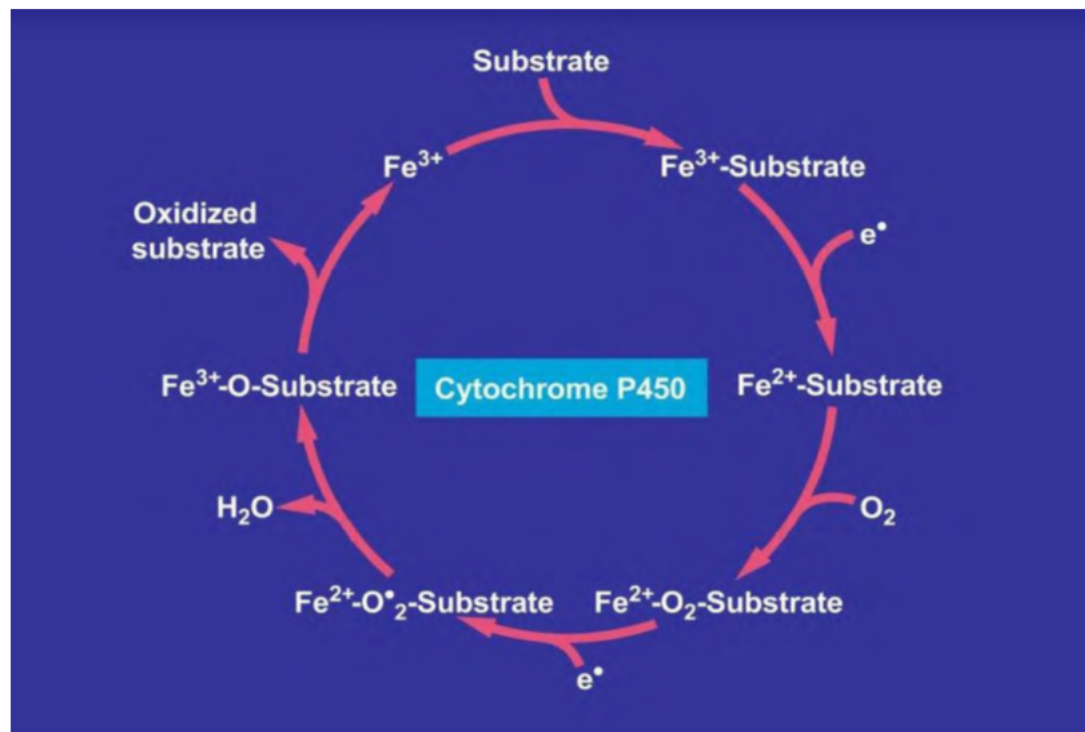

Figure 2: *Generation of ROS through the cytochrome P450 cycle. Substrates entering the cytochrome P450 cycle will be oxidized, and reactive oxygen species will additionally be generated. Figure is derived from a previous report [59].*

Endotoxins and the gut-liver axis in ALD

Produced from intestinal bacteria, endotoxins enter the liver via the portal circulation [4,10]. Chemically known as lipopolysaccharides (LPS), endotoxins play a contributory but by no means a permissive role for initiation and perpetuation of ALD [4,10,60-62], because a variety of other mechanistic events previously been described as causative in detail [1-4].

Endotoxins are commonly found increased in the serum of patients with ALD [4,62] if clearance through the liver is inadequate [62]. Endotoxins then gain easily access to the hepatocytes and non-parenchymal cells that may be stimulated and release active mediators including cytokines [10,62]. In essence, several cells and various mechanistic steps are essential for promoting liver injury, illustrated as hypothesis (Figure 3).
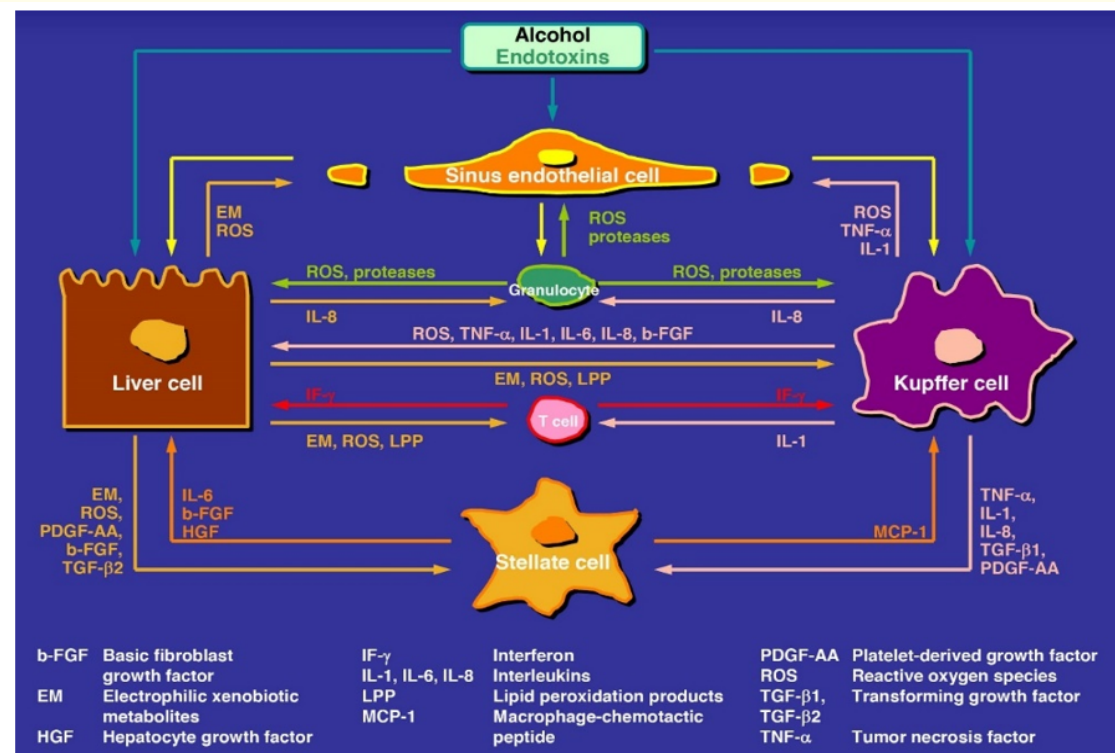
Figure 3: *Hypothetical actions of endotoxins on the liver cell and non-parenchymal cells of the liver. Tentative steps leading to early and intermediate stages of alcoholic liver disease. The pathogenesis involves various mediators and cell types of the liver, some of the steps need confirmation and are therefore hypothetical. The original figure was published in a recent report [8] and is reproduced with permission of the Publisher Taylor and Francis (Didcot, UK).*

More specifically, the liver actually responds to the LPS chemicals, which initiate liver injury through mechanisms involving inflammatory cells like Kupffer cells and via chemical mediators such as superoxide radicals, nitric oxide, and tumor necrosis factor (TNFα) [62]. Ethanol has been shown to modify several functions of Kupffer cells, and its prolonged use caused a 7-fold increase of metabolically active CYP 2E1 in Kupffer cells [60]. This upgrading of CYP 2E1 will enhance the production of cytokines, ROS, and acetaldehyde from ethanol metabolism [4,10,62]. Therefore, within the Kupffer cells present in the alcoholic liver, variable interactions can be assumed, leading to local disturbances within these cells and to alterations of cellular pathways affecting the liver in general, although the sequence of events is yet poorly understood and myriads of proposals have been presented [62]. Good experimental evidence exists that activated Kupffer cells can stimulate hepatic stellate cells; these become a major source of enhanced production of extracellular matrix proteins that initiate fibrosis [4]. Not yet studied in detail is also the situation of ROS, CYP 2E1 in hepatic stellate cells and possible interactions with endotoxins [61]. Overall clinical and experimental data support the concept of ALD that the liver injury is closely related to modifications observed in the intestinal tract and helps establish a gut-liver axis to explain mechanistic events related to ALD [4,10-13,60-64].

What is even more, the intestinal microbiome is considered to play a pathogenetic role promoting various diseases unrelated to ALD [65-67]. Among these are intestinal disorders such as Crohn's disease, ulcerative colitis, irritable bowel syndrome, and celiac disease [65], heart diseases [65,66], cancer [65], and others like autism, Alzheimer's disease, multiple sclerosis, Parkinson's disease, and rheumatoid arthritis [65]; of clinical interest are nonalcoholic fatty liver disease [65,67] and nonalcoholic steatohepatitis [67] both liver disease types are closely connected to overweight, obesity, or morbid obesity [65,67,68], as well as to insulin resistance, metabolic syndrome and diabetes mellitus [68].

With respect to the gut, the intestinal microbiome plays an essential role in ALD [63,64], whereby microbiome may be defined as a community of microorganisms that include bacteria, fungi, and viruses living in a predefined part of the human body [4,10-13,60-65]. Initially known as intestinal microflora, it has now been renamed intestinal microbiota because of the diversity of microorganisms that are far away from plants based on genomic studies [65].

Intestinal microbiota are commonly studied in fecal specimens and represent mostly microbes of the colon because healthy humans do not harbor microbes in the small intestine. In patients with a history of alcohol abuse, studies on jejunal aspirates revealed bacterial overgrowth, with preference of Gram-negative anaerobic bacteria and endospore-forming rod bacteria [14]. Endotoxins are derived from the outer membrane of Gram-negative intestinal bacteria, are increasingly produced due to bacterial overgrowth in the small intestine, and enter in increasing amounts the portal vein due to a leaky intestinal mucosa [13,64].

Several mechanisms may contribute to this increased gut leakage, including a direct injurious effect of the ingested alcohol on the integrity of the intestinal mucosa, and an increased production of ROS and acetaldehyde due to induction of CYP 2E1 and MEOS activity in the intestinal mucosa by prolonged alcohol abuse [10,64]. Gut leakage and endotoxemia can also be increased by binge alcohol use [10] and may be modified by circadian rhythms, based on a novel CYP 2E1-circadian clock protein mechanism [64]. Thus, sufficient data exist in support of the intestinal microbiome and gut-liver axis hypothesis that endotoxins produced by intestinal bacterial overgrowth cross the leaky intestinal mucosa and contribute to hepatic apoptosis and steatohepatitis, which is still a major clinical issue and merits further discussions on potential new specific therapeutic approaches.

Conclusions

Evidence is accumulating that in addition to previous molecular and mechanistic concepts with focus on liver injury, the intestinal microbiome may be a contributory factor for initiation and aggravation of alcoholic liver disease. With respect to the intestinal microbiome and the resulting gut-liver axis, pathogenetic considerations currently focus on intestinal bacterial overgrowth, increased gut leakage for bacterial endotoxins, which in turn may injury the liver through signaling mediators produced by specific cells including Kupffer cells and Stellate cells. Gut leakage may be increased by induction of MEOS activity and CYP 2E1 in the intestinal mucosa, resulting in enhanced metabolism of ethanol to the toxic acetaldehyde and ROS. Other modifiers are binge type of alcohol use and circadian rhythms. It remains to be established whether these developments of intestinal microbiome may result in future therapeutic options.

Conflict of Interest Statement

The authors declare that they have no conflict of interest with respect to this invited article.

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