Alcohol, Inflammation, and Microbiota in Alcoholic Liver Disease

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Alcoholic liver disease (ALD) is a consequence of excessive alcohol use. According to many studies, alcohol represents a significant socioeconomic and health risk factor in population. According to data from the World Health Organization, there are about 75 million people who have alcohol disorders, and it is well known that its use leads to serious health problems. ALD is a multimodality spectrum that includes alcoholic fatty liver disease (AFL) and alcoholic steatohepatitis (ASH), consequently leading to liver fibrosis and cirrhosis. In addition, the rapid progression of alcoholic liver disease can lead to alcoholic hepatitis (AH). Alcohol metabolism produces toxic metabolites that lead to tissue and organ damage through an inflammatory cascade that includes numerous cytokines, chemokines, and reactive oxygen species (ROS). In the process of inflammation, mediators are cells of the immune system, but also resident cells of the liver, such as hepatocytes, hepatic stellate cells, and Kupffer cells. These cells are activated by exogenous and endogenous antigens, which are called pathogen and damage-associated molecular patterns (PAMPs, DAMPs). Both are recognized by Toll-like receptors (TLRs), which activation triggers the inflammatory pathways. It has been proven that intestinal dysbiosis and disturbed integrity of the intestinal barrier perform a role in the promotion of inflammatory liver damage. These phenomena are also found in chronic excessive use of alcohol. The intestinal microbiota has an important role in maintaining the homeostasis of the organism, and its role in the treatment of ALD has been widely investigated. Prebiotics, probiotics, postbiotics, and symbiotics represent therapeutic interventions that can have a significant effect on the prevention and treatment of ALD.

Keywords: alcohol ; inflammation ; microbiota ; alcoholic hepatitis

1. Alcohol Metabolism

Alcohol metabolism takes place mostly in the liver [1]. It begins under the influence of alcohol dehydrogenase, and in the presence of oxygen and coenzyme NADPH. In this way, a highly reactive and toxic metabolite called acetaldehyde is formed [1]. The reaction of acetaldehyde synthesis takes place in the cytosol of hepatocytes, and it is further metabolized under the influence of acetaldehyde dehydrogenase in the mitochondria to acetate. The well-known cytochrome P450 2E1, or CYP2E1, which is located in the endoplasmic reticulum and mitochondria, participates in alcohol metabolism [1][2]. Its activation by chronic alcohol use leads to the formation of reactive oxygen species (ROS). This is how the so-called alcohol-induced inflammation occurs. Evidence for this is that inhibition of CYP2E1 by chlormethiazole improves ALD and reduces the carcinogenic effect of acetaldehyde in experimental animals [3][4]. In addition, the so-called microsomal ethanol-oxidizing system (MEOS) was defined and discovered in the middle of the last century by C. S. Lieber and L. M. DeCarli [5]. Chronic excessive use of alcohol leads to the acceleration of alcohol metabolism via MEOS, precipitating the formation of toxic metabolites and faster tissue and organ damage [5]. Acetaldehyde is toxic and carcinogenic and leads to structural and functional damage to cell organelles [6].

They further lead to changes in the structure of protein molecules, lipid peroxidation, and damage to DNA molecules [7][8]. In addition, they can lead to post-translational modifications, such as methylation, phosphorylation, and acetylation. Accumulation of ROS subsequently change macromolecules causing the occurrence and progression of already existing liver damage [8].

2. Pathogen-Associated Molecular Patterns (PAMPs), Damage-Associated Molecular Patterns (DAMPs), Toll-like Receptors (TLRs), and Inflammation

Drinking triggers inflammation through two types of molecules called pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) [9][10]. Both are recognized by pattern-recognition receptors (PRRs),

and thus, the inflammatory cascade begins. The most famous of this group of receptors are Toll-like receptors (TLRs), of which there are several classes. They can recognize both exogenous and endogenous pathogens and are expressed by both immune and liver parenchymal cells [11].

PAMPs are products of microorganisms that reach the liver via the lymphatic system and portal circulation. In the case of Gram-negative bacteria, it is lipopolysaccharide (LPS), which has been proven to be one of the best stimulators of TLRs [12]. In the case of chronic excessive use of alcohol, the permeability of the intestinal barrier increases, and the translocation of intestinal microbiota products takes place more easily into the lymph flow and portal circulation [13]. In this way, PAMPs reach the liver, where they activate Kupffer cells, which then activate infiltrating monocytes, stellate, and other cells. As a result of these activities, cytokines and chemokines that characterize the inflammatory response are produced. PAMPs most often through TLR4 cause NF-κβ activation and the release of CC-chemokine ligand 2 (CCL2) and IL-8, which then cause infiltration of the liver by macrophages and neutrophils [13]. In addition, TNF and IL-6 are released, which also have a pro-inflammatory effect. Their increased concentration is measured in acute AH and is associated with a worse outcome [13]. On the other hand, in the absence of infection, so-called sterile inflammation occurs, and it is characterized by integrity damage and death of cells [14]. On this occasion, DAMPs are released, most commonly ATP, adenosine, DNA, uric acid, heparin sulfate fragments, and others. Alcohol by its direct toxic effect promotes cell death in various ways, predominantly by activating the mitochondrial apoptotic program and damaging the endoplasmic reticulum [15]. This phenomenon is most pronounced in AH [16].

After the activation of TLRs, the post-translational processing of the pro-forms of cytokines and the release of their active forms occurs, e.g., IL-8 which has already been discussed and IL-1β. IL-1β acts through the TLR4 receptor by activating NF-κβ as previously reported [13]. Its active form is released only after the activation of a complex called the inflammasome [17]. Inflammasome is a multiprotein complex that belongs to the innate immune response and activates caspase-1, the effect of which, among other things, creates the active form of IL-1β from the inactive one [17][18]. This cytokine has a pro-inflammatory effect, autocrinely increasing its concentration and stimulating the release of TNF. This process increases the sensitivity of hepatocytes to apoptotic factors [19][20]. Research has shown that by blocking the receptor for IL-1β, the regeneration of hepatocytes is stimulated [17]. Additionally, IL-1β promotes liver fibrosis by activating hepatic stellate cells via matrix metallopeptidase 9 (MMP9) [21]. This form of the inflammasome, which involves the activation of caspase-1, is called the canonical inflammasome. Non-canonical inflammasome includes the CASP4/11-GSDMD complex, which activation causes cell death through lysis [22][23]. It is very important in infection-induced cell death with the consequent release of DAMPs and the initiation of an inflammatory response. This reaction can be so intense that it triggers systemic inflammatory response syndrome (SIRS), which can be seen in the rapid progression of ASH to AH. This often happens in infections caused by Gram-negative bacteria when LPS activates the CASP4/11-GSDMD pathway, the activity of proteolytic enzymes is triggered inside hepatocytes, then the release of DAMPs and pro-inflammatory cytokines. If hepatic macrophages and stellate cells succumb to cell lysis, systemic dissemination of endotoxin occurs with the development of SIRS and sepsis [9][22][23].

3. What Is the Role of Intestinal Microbiota in the Mentioned Processes?

In the gastrointestinal tract, there are more than a trillion microorganisms, primarily bacteria, but also viruses, fungi, protozoa, and archaea. They participate in digestion, metabolism of various substances, and endogenous production of alcohol, but also in host immunity [24]. Endogenous production of alcohol by bacteria of intestinal microbiota implies a fermentation process that takes place independently of the exogenous introduction of alcohol. This has been proven, for example, in an obese mouse model [25]. The endogenous production of alcohol has been proven by measuring the level of alcohol in the blood of people who have not consumed it in a certain period. Bacteria in anaerobic conditions switch to a mixed-acid fermentation pathway, and the main product of this pathway is alcohol [26]. Alcohol produced in this way is also metabolized by alcohol dehydrogenase, which is most active in the liver and gastrointestinal tract [27]. It is interesting that Zhu et al., showed that the level of endogenous alcohol in the blood is higher in obese patients with proven non-alcoholic steatohepatitis than in obese patients who do not have it. These results are explained by differences in the composition of the microbiota [27].

The connection between the intestine and the liver is bilateral: microorganisms and their products reach the liver through the lymph flow and portal blood flow, and on the other hand, the bile produced in the liver through the biliary tract is secreted into the intestine. Alcohol consumption causes changes in the composition of the intestinal microbiota, making it less diverse, reducing the number of bacteria that have a beneficial effect on health, and increasing the number of those that can have a harmful effect. This refers primarily to Gram-negative bacteria [28]. In addition, chronic excessive use of alcohol increases the possibility of overgrowth of Candida species [29]. The intestinal barrier is the first point of defense of the organism against the penetration of pathogenic microorganisms and their products into circulation [30]. It possesses

components of the innate and acquired immune system, such as neutrophils, secretory immunoglobulins, and T lymphocytes. Its integrity is impaired in alcoholics [30]. According to some data, more than half of alcoholics have proven intestinal barrier dysfunction and intestinal dysbiosis [31]. This increases the possibility of translocation of products of microorganisms, but also of live bacteria, into the systemic circulation, which will reach the liver. This phenomenon increases the possibility of ALD [31]. Increased permeability of the intestinal barrier, or the so-called “leaking gut”, is caused by the effect of alcohol on the tight junctions that are connected to the intestinal epithelial cells [32].

The intestinal microbiota is very diverse, and its composition differs depending on the part of the digestive tract and the number of bacteria it contains. For example, the colon has the richest microbiota [33]. Two main families of bacteria make up the intestinal microbiota, namely *Firmicutes*, as a representative of Gram-positive bacteria, and *Bacteroidetes*, which belong to Gram-negative bacteria [34][35]. The share of these two families in the composition of the intestinal microbiota is individual and differs in many conditions [36]. Apart from the two mentioned, several smaller families contribute to the diversity of the intestinal microbiome. Less than 0.1% of the microbiome consists of potentially pathogenic bacteria, such as *Escherichia coli*, *Campylobacter jejuni*, and *Bacteroides fragilis*. It has been proven that intestinal dysbiosis can perform a role in the development of inflammatory bowel diseases (IBD), autoimmune and metabolic disorders, and so on [37][38].

In ALD, the existence of small intestinal bacterial overgrowth (SIBO) and a decrease in the number of bacteria from *Lactobacillus* species have been proven [39][40]. They produce bactericidal substances that maintain the homeostasis of the intestinal microbiota and prevent the reproduction of pathogenic bacteria, such as *Salmonella* and *Shigella* species. Short-chain fatty acids (SCFAs), which include butyrate, acetate, and propionate, are produced as products of their metabolism. The role of SCFAs is to maintain the integrity of the intestinal barrier, and, in addition, they are a source of energy for intestinal epithelial cells and have an immunological role [41][42][43].

The interaction between the intestine and the liver is bilateral. Bile acid is important in the pathogenesis of liver diseases, in which intestinal dysbiosis also performs a significant role. Bile acids perform a role in fat emulsion [44]. In addition, they are signaling molecules that bind to G protein-coupled receptors and regulate lipid and glucose metabolism. In hepatocytes, primary bile acids are conjugated, which allows them to be reabsorbed at the level of the terminal ileum. A smaller part is not a subject to this recirculation, and, in the distal parts of the intestine, are converted into secondary bile acids under the influence of bacteria through the processes of hydroxylation and esterification [44]. Additionally, bile acids affect the composition of intestinal microbiota. *Clostridium*, *Lactobacillus*, *Bifidobacterium*, and others have an enzyme that deconjugates primary bile acids and enables their excretion through the digestive tract. Bile acids can have a protective role, i.e., prevent intestinal barrier dysfunction and maintain gut eubiosis. Secondary bile acids are more toxic to intestinal and hepatic cells, and studies have shown that their concentration is elevated in the feces and serum of alcoholics [45]. Intestinal epithelial cells and hepatocytes express the farnesoid X receptor (FXR) that recognizes the mentioned acids. Upon binding and activation of the receptor, the endocrine hormone fibroblast growth factor (FGF 19) is released, which inhibits the de novo synthesis of bile acids. However, when there is intestinal dysbiosis, the level of FGF 19 decreases, and an increase in bile acid synthesis occurs, thus secondary toxic bile acids are produced in a higher percentage than primary ones [46].

4. Therapeutic Interventions in Alcoholic Liver Disease (ALD) Involving the Microbiota

Given the importance of gut microbiota homeostasis in the pathogenesis of ALD, therapeutic options targeting it seems promising. It has been proven that the use of prebiotics, probiotics, postbiotics, and symbiotics can lead to the improvement of chronic liver diseases, ALD, and liver cirrhosis among others [47].

Probiotics are a group of non-pathogenic microorganisms whose role is to modulate and maintain homeostasis of the intestinal microbiota. They can reduce inflammation in the case of alcohol-induced liver inflammation and prevent “leaky gut”. For example, this is demonstrated using #BCL3 which includes Bifidobacterium breve, B. infantis, B. longum, L. acidophilus, L. paracasei, L. bulgaricus, L. plantarum, and Streptococcus thermophilus [48][49][50]. Prebiotics are food ingredients that are not digestible and help intestinal peristalsis and stimulate the growth of certain bacteria. Some studies have shown that prebiotics repair alcohol-induced liver damage in a mouse model by reducing bacterial overgrowth. Postbiotics or microbe-derived metabolites can also be used in the treatment of ALD [49][51].

Fecal microbiota transplantation (FMT) involves the transplantation of part of the microbiota of a healthy person to a person suffering from certain diseases. For example, the role of FMT in patients with AH and liver cirrhosis was investigated and satisfactory results were obtained. In a study conducted by Bajaj et al., 20 patients with liver cirrhosis of

the most common viral etiology (predominantly HCV) with or without data on chronic alcohol use were included. The patient received a single FMT enema with or without antibiotics. It was concluded that FMT is a safe and well-tolerated procedure, it increases the diversity of intestinal microbiota and the number of beneficial bacteria. Patients who had FMT had significantly lower chance of developing AH [52]. Another study followed the 1-year survival of patients with AH who had FMT, where as many as 87.5% of patients who had FMT survived one year, compared to 33.5% of patients who were controls [53].

A promising approach in the treatment of ALD is also an attempt to rehabilitate the previously disturbed homeostasis of bile acid metabolism, which was proven in the mouse model of ethanol-induced liver damage. Return of homeostasis using a non-tumorigenic variant of FGF19 improves intestinal barrier function and reduces liver damage. Obeticholic acid (OCA) is an FXR agonist and has been proven to prevent intestinal vascular barrier disorders, which is why it has a potential place in the treatment of nonalcoholic fatty liver disease (NAFLD), while there is still insufficient data for ALD [54] [55].

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