Targeting the gut microbiota to treat alcoholic liver diseases : evidence and promises

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Abstract

The human intestine is colonized by a variety of microbes that influence the metabolic responses, the immune system and the nervous system. Dietary patterns are important factors that shape the composition of the gut microbiota. Many animal models of alcohol exposure have highlighted the key role of the alcohol-induced gut microbiota alterations, leaky gut and translocation of microbial products in the development of alcoholic liver disease (ALD). However, in humans, there is no clear picture defining an "alcoholic microbiome", and the link between intestinal dysbiosis and ALD development is far from being understood. Although we do not comprehend all the mechanistic insights, clinical studies aiming at modulating the gut microbiota of alcoholic patients have shown some beneficial effects. Here we review the potential therapeutic effects of probiotics in ALD and give some clinical perspectives on the role of prebiotics and the use of fecal microbiota transplantation. (Acta gastroenterol. belg., **2020**, 83, **000-000**).

Key words : gut microbiota, alcoholic liver disease, probiotics, prebiotics, fecal microbiota transplantation, metabolomics.

The gut microbiota

The human intestine is colonized by a variety of microbes (bacteria, viruses, fungi and archeae) (1,2). The number of bacterial cells in the gut is approximatively similar to the number of human cells in the body, but the human microbiome contains over 3 million genes, compared with 23.000 genes in the human genome (3). Most of the intestinal bacteria belong to 2 phyla, the Firmicutes (gram positive) and the Bacteroidetes (gram negative), while the rest belongs to Actinobacteria, Fusobacteria, Proteobacteria and Verrucomicrobia. Each phyla is then divided into class, order, family, genus, species of bacteria. The human gut microbiota is constituted by 500-1000 bacterial species which maintain a symbiotic relationship with the host, and play crucial role in metabolic, immune and neurobiological responses (4). It also helps in the maintenance of the intestinal barrier function, which limits pathogen invasion, synthesizes a variety of metabolites, vitamins and neurotransmitters, metabolizes bile acids and influences drugs metabolism. The presence and abundance of intestinal bacteria are mostly shaped by dietary patterns (5,6). However, external factors like the use of antibiotics, other medications (proton-pump inhibitors, metformin, laxatives, antidepressants such as selective serotonin reuptake inhibitors (SSRI)) and other life style perturbations such as stress can profoundly influence the composition of the human microbiome (4,7).

The gut microbiome and metabolome in patients with alcohol use disorders

The gut microbiota composition can be assessed through different techniques, ranking from stool culture to next-generation 16S rDNA gene sequencing or shotgun metagenomics approaches (8). Several studies have interrogated the gut microbiota of alcohol use disorder (AUD) patients with or without alcoholic liver disease (ALD). They led to conflicting results (see review (9)) and no clear picture defining an "alcoholic microbiome" has emerged from those efforts probably due to the high heterogeneity of patients, the drinking status (actively drinking vs. short-term or long-term abstinence), the stage of liver disease (steatosis, fibrosis, steatohepatitis, cirrhosis), different dietary patterns, medications used, the biological material (stool samples vs duodenal or colonic biopsies), and the different techniques and bioinformatics pipelines used to analyze the bacterial composition (Figure 1). Furthermore, to identify potential bacteria involved in ALD development, the reference to a healthy control group without a history of alcohol abuse and ALD is needed but the microbial features of a "healthy microbiota" are still a matter of debate (10). Authors usually refer to the term "dysbiosis' to describe imbalance or alterations of the microbiota that can have unfavorable effects on the host. Common features of dysbiotic microbiota of alcoholic patients include lower Bacteroidetes, lower Ruminococcaceae including the anti-inflammatory bacterium *Faecalibacterium prausnitzii*, higher Proteobacteria, Lachnospiraceae and Enterobacteriaceae, changes in *Lactobacillus*, *Bifidobacterium*, *Akkermansia* (11-16). Interestingly, recent studies showed

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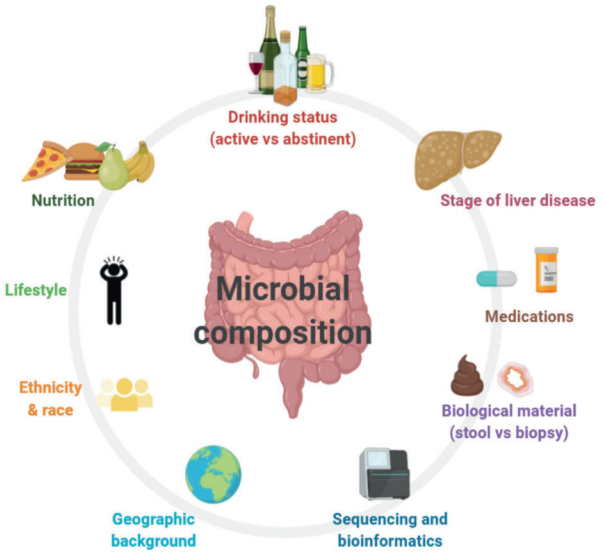

Figure 1.

overrepresentation of microbial species originating from the oral microbiota in the stool or duodenal biopsies of ALD patients such as *Streptococcus, Shuttleworthia* and *Rothia* (13,16) (see Table 1).

Many animal models of alcohol exposure have highlighted the key role of the alcohol-induced leaky gut and translocation of microbial products with potent pro-inflammatory agents (e.g. lipopolysaccharides, peptidoglycans) in the development of ALD (17). However, the observations made in mice and rats cannot easily be extrapolated to human alcoholic pathology likely due to multiple factors such as the natural aversion of rodents for alcohol, the differences in immune systems and in the higher metabolism of ethanol in rodents (18). Indeed, several studies have shown an increased intestinal permeability and alterations of the fecal or colonic mucosal microbiota only in a subset of AUD patients and those alterations where independent of the degree of liver disease (11,12,16). Interestingly, we demonstrated for the first time that alterations in the duodenal mucosa-associated microbiota - but not fecal microbiota-, were associated with liver disease progression (16).

While it is almost impossible to identify microbial taxa responsible for the development of ALD in human pathology, it might be more relevant to interrogate the metabolome - which represents the large catalogue of metabolites produced or to some extent influenced by the gut microbiota. Untargeted metabolomics analysis of stools, urine or blood, using mass spectrometry or nuclear magnetic resonance spectroscopy approaches, are useful to gain pathophysiological insight into the disease process by identifying potential key metabolites involved in ALD. Among them, altered levels of short chain fatty acids (SCFAs), long chain fatty acids, bile acids, dimethyl sulfide, dimethyl trisulfide, indole compounds, phenol, cytolysin - an exotoxin secreted by *Enterococcus feacalis*-, fungal β-glucan and candidalysin - a toxin secreted by *Candida albicans* - have been observed in animal models and AUD patients with different degrees of liver diseases ((11,19-21) and for review see (22)). Hence, not only bacterial but also fungal metabolites related to the intestinal mycobiome could be involved in the development of ALD. Recently, a decrease in fungal diversity and an overgrowth of *Candida* species have been observed in AUD patients (23).

Consequently, innovative approaches that can reduce the extent of alcohol-induced injury by restoring the gut microbiome, the gut mycobiome, the associated metabolic pathways or nutritional interventions might be promising (24). Here we review the potential therapeutic effects of probiotics in ALD and give some clinical perspectives on the role of prebiotics and the use of fecal microbiota transplantation.

Probiotics definitions and action mechanisms in ALD

The scientific definition of the term ‘probiotic’ was proposed in 2001 by a joint report of the FAO/WHO, and confirmed in 2013 by an expert panel of ISAPP

Table 1. — **Non-exhaustive list of gut microbiota alterations in AUD patients**

Microbial taxa over-represented in AUD patients	Microbial taxa under-represented in AUD patients	Microbial taxa with controversial results
Proteobacteria (12)	Bacteroidetes (12)	*Bifidobacterium* (11,13,14)
Lachnospiraceae (11)	Ruminococcaceae (11)	*Akkermansia* (13,15,16)
Streptococcus (13,14,16)	*Alistipes* (13)	
Enterobacteria (14)	*Coprococcus* (13)	
Klebsiella (13)	*Paraprevotella* (13)	
Lactococcus (13)	*Prevotella* (13)	
Lactobacillus (13)	*Faecalibacterium praustnizii* (11,13)	
Shuttleworthia (16)		
Rothia (16)		
Gemella (16)		
Actinomyces (16)		
Desulfovibrio (16)		
Candida albicans (23)		
Candida dubliniensis (23)		

AUD : alcohol use disorder

Table 2. — **Clinical trials using probiotics in alcoholic liver disease**

Study	Population, stage of liver disease and drinking status	Treatment, dose and duration	Microbiota assessment technique	Microbiota outcomes	Liver outcomes	Other outcomes
Kirpich et al. 2008 (29) Single center, open-label, randomized, prospective study	66 adult Russian males with mild alcohol-induced liver injury Subgroup of 26 subjects with well-characterized alcoholic hepatitis (AH) Last drink within 48h of admission	Group 1 (n = 34): standard therapy alone (abstinence + vitamins) Group 2 (n = 32): standard therapy + probiotics (0.9×10^8 CFU *Bifidobacterium bifidum* and 0.9×10^9 CFU *Lactobacillus plantarum 8PA3 administered once daily during 5 days)*	Stool culture	5-day probiotic therapy vs standard therapy: Increase in *Bifidobacterium Lactobacillus Enterococcus*	5-day probiotic therapy vs standard therapy: Greater reduction of AST and ALT activity 5-day probiotic therapy vs standard therapy in the subgroup of AH patients: Greater reduction of ALT, GGT, lactate dehydrogenase, and total bilirubin	
Stadlbauer et al., 2008 (30) Single center, open-label study, not randomized	20 outpatients with compensated alcoholic cirrhosis (males and females) All patients were abstinent for at least 1 months prior and during the study	Group 1 (n = 8): no treatment Group 2 (n = 12 but 2 patients lost of follow-up): Patients received food supplementation with Yakult® *(Lactobacillus casei* Shirota) 3 times daily for 28 days. Dose of bacteria = 1.95×10^{10}/day.				Phagocytic capacity of neutrophils was increased after 4 weeks of probiotic therapy
Koga et al 2012 (31) Single center, randomized, double blind, placebo controlled study	37 hospitalized patients with compensated alcoholic cirrhosis (Child-Pugh grade A) (males and females) No information on drinking status	Placebo group 1 (n = 19): patients received beverages containing lactic acid Probiotic group (n = 18): patients received beverages containing Y400 (*Lactobacillus casei* Shirota YIT 9029 – dose $=4\times 10^{10}$ CFU/serving) Beverages (80 mL, 62 kcal) were administered twice a day for the first 2 weeks of the 4-week study	Real-time quantitative PCR	Probiotic group vs placebo: Higher number of Clostridum coccoides and *Eubacterium cylindroides* (at week 2) Lower number of *Enterobacteriaceae* (at week 4)	Probiotic group vs placebo: Higher serum levels of transthyretin and albumin (p = 0.08) at week 3 Lower level of hsCRP at week 4 No difference in AST, ALT, GGT, total bilirubin	
Han et al (2015) (32) Multicenter, randomized, double blind, placebo controlled, clinical trial	117 hospitalized patients with mild alcohol hepatitis (males and females) (53% of patients had liver cirrhosis) Last drink within 48h of admission. Patients were abstinent during the 7-day study	Placebo group (n = 57): no information about the placebo Probiotic group (n = 60): patients received cultured *Lactobacillus subtilis/ Streptococcus faecium* (1500 mg/day) 3 times a day, during 7 days Both groups were supplemented with Legalon® capsules as standard therapy	Stool culture	Probiotic group vs placebo: Decrease in *Escherichia coli*	Increase in serum LPS after 7 days only in the placebo group No difference in AST, ALT, GGT, total bilirubin between probiotic and placebo groups	Probiotic group vs placebo: Decrease in serum TNFα

AH, alcoholic hepatitis ; AST, aspartate aminotransferase ; ALT, alanine transaminase ; CFU, colony forming unit ; GGT, Gamma-glutamyltransferase ; hsCRP, high-sensitivity C-reactive protein ; LPS: lipopolysaccharide.

(25). The currently accepted definition of probiotics is : "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host". While mechanisms of action of probiotics have sometime been described extensively, in most cases, despite a well demonstrated health benefit, those mechanisms are still unknown. A common misconception is that probiotics need to change the gut microbiota composition in order to be effective. Actually, probiotics are not known to take up permanent residency in the gut, and they rapidly disappear after treatment cessation (26).

Most probiotics contain *Lactobacillus* and *Bifidobacterium* which are saccharolytic bacteria that can ferment carbohydrates and produce SCFAs including acetate, propionate and butyrate. These SCFAs are known to inhibit the growth of pathogens, reinforce the gut barrier function, exert anti-inflammatory actions (27). In the context of ALD, some potential mechanisms of action of probiotics were identified thanks to animal studies. Rats exposed to alcohol for 10 weeks and treated with *L. rhamnosus GG* exhibited less severe hepatic damage, lower intestinal permeability, lower oxidative stress and inflammation in both intestine and liver compared to rats treated with alcohol and the vehicle (28). In mice, *L. rhamnosus GG* culture supernatant was effective in the prevention of chronic alcohol exposure-induced hepatic steatosis and injury through the modulation of liver AMPK phosphorylation and Bax/Bcl-2-mediated apoptosis (29). Finally, *Akkermansia muciniphila* administration prevented neutrophil infiltration and lowered hepatic steatosis and injury in a mouse model of alcohol-related liver disease (15).

Clinical trials using probiotics in ALD

The number of well-conducted clinical trials targeting patients with liver disease due to alcohol abuse is very limited and summarized in Table 2. The first human pilot study demonstrating a therapeutic role for probiotics in the short-term treatment of ALD has been conducted in 2008 by Kirpich *et al* (30). The authors demonstrated that a 5-day probiotic therapy during alcohol detoxification was associated with a greater improvement in liver enzymes (serum transaminases) than abstinence alone in patients with mild ethanol-induced liver injury/alcoholic hepatitis. The improvement of liver function tests was associated with increased fecal level of *Bifidobacterium* and *Lactobacillus*. Another study showed that the phagocytic capacity of neutrophils was restored in patients with alcoholic cirrhosis receiving 1 month of probiotic therapy (31). In 2012, Koga *et al*. conducted a randomized, double blind, placebo controlled clinical trial to test the effects of *Lactobacillus casei* Shirota on liver function in patients hospitalized with alcoholic cirrhosis (32). The results of this study showed that the conventional liver function tests improved during the 4-week hospitalization but did not differ between the probiotic and placebo groups. However, the serum level of the nutritional marker transthyretin, secreted by hepatocytes and involved in retinoid metabolism occurring in hepatic stellate cells (HSCs), was increased in the probiotic group, and that could be beneficial to reduce the progression of liver fibrosis by inactivating HSCs. Probiotic therapy also decreased the serum levels of hsCRP and modified fecal levels of some bacterial taxa (*Clostridum coccoides*, *Eubacterium cylindroides, Enterobacteriaceae*). Finally, Han et al showed in a multicenter, randomized, double blind, placebo controlled study that a 7-day supplementation with cultured *Lactobacillus subtilis/Streptococcus faecium* reduced serum TNFα and LPS in alcoholic hepatitis patients (33).

Prebiotics and fecal microbiota transplantation in ALD

The new definition of prebiotics is "a substrate that is selectively utilized by host microorganisms conferring a health benefit" (34). The previous definitions only included carbohydrates-based substrates such as fructooligosaccharide, galactoolisaccharide and inulin but in 2016, the definition of prebiotic has been extended to include other substances such as polyphenols and polyunsaturated fatty acids. Furthermore, it is recognized today that the prebiotic effect probably extends beyond *Bifidobacterium* and *Lactobacillus* since the growth of other beneficial bacterial taxa, such as *Faecalibacterium*, *Roseburia*, *Eubacterium* can also be promoted.

In a mouse model of alcohol-induced liver disease, treatment with fructooligosaccharides partially restored the antimicrobial Reg3g protein levels, reduced bacterial overgrowth, and lessened alcoholic steatohepatitis (35). More recently, the prebiotic pectin treatment in mice prevented alcohol induced-steatosis, liver inflammation, and restored gut homeostasis (36). The first clinical trial assessing the impact of prebiotics supplementation on gastrointestinal tolerance and on the gut-liver-brain axis modulation of AUD patients undergoing alcohol detoxification is currently ongoing at St-Luc academic Hospital (NCT03803709).

In the past few years, the fecal microbiota transplantation (FMT) has been considered an option in the treatment of human diseases, with an impressive track record of success curing *Clostridium difficile* infections (37) and better clinical remission rate in ulcerative colitis (38). In preclinical models, investigators found that fecal microbiota transfer from human AUD patients to mice led to transmissibility of alcohol-related liver disease, as well as alcohol-related behavioral alterations. Indeed, Llopis *et al*. showed that mice transplanted with the fecal microbiota of patient with severe alcoholic hepatitis developed more severe liver inflammation and necrosis, greater intestinal permeability and bacterial translocation compared to mice transplanted with the fecal microbiota of alcoholic patient without hepatitis (14). In addition, we showed for the first time that mice harboring a

human dysbiotic alcoholic microbiota exhibited higher depression-like behavior and reduced social behavior compared to mice harboring a human healthy microbiota (39).

There are very limited FMT studies in ALD patients. Philipps *et al*. conducted a 1-year follow-up pilot FMT study on 8 patients with steroid-resistant severe alcoholic hepatitis and showed an overall improvement of liver function and survival rate of the recipient patients compared to patients who did not receive FMT (87.5% vs 33.3%) (40). Higher survival rate were also observed by the same investigators in another cohort of 16 patients receiving FMT who were compared to patients treated with current therapies (corticosteroids, nutritional support or pentoxifylline) (41). In 2020, the first double-blind, placebo controlled, randomized clinical trial on FMT including 20 AUD-related cirrhosis patients was published by Bajaj *et al* (42). The results showed a reduction of alcohol craving, improvement of cognition and psychosocial parameters, as well as a reduction in serum level of IL-6 and LBP, a marker of microbial translocation, in the FMT group. Modifications of the gut microbiota composition (higher Ruminococcaceae) and function (increased SCFAs) were also observed post-FMT. At 6 months, the number AUD-related serious adverse events was higher in the placebo group. The mechanisms of action of FMT are not yet understood but it seems that the donor microbiota does not totally replace but rather modifies the bacterial species present in the recipient host, leading to a symbiotic coexistence.

Conclusion and future directions

Numerous experimental models demonstrated that the gut microbiota is intricately involved in the development of alcoholic liver disease although convincing evidence in humans is lacking. Translational studies with focus on the upper-part of the intestine, rather than the colon, might bring new insights and evidence linking the gut to liver disease progression as well as to alterations in the brain perpetuating behavioral changes and alcohol seeking in AUD patients. Large and long-term clinical studies assessing the modulation of gut microbiota or its associated metabolites, though specific microbial consortia, prebiotics supplementation or via fecal microbiota transplantation represent a promising area of research in the context of the gut-liver-brain axis in patients suffering from alcohol use disorders and for whom current therapeutic options are limited.

Conflict of interest

The authors have no conflict of interest to disclose.

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