Viewpoint

Are probiotics effective in reversing non-alcoholic steatohepatitis?

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The gut microbiota (GM) is an integrated ecosystem of tens of trillions of microorganisms, as actinomyces, archaea, protozoa, and last but not least viruses, including one thousand different species of known bacteria with an overall count of more than three million genes, 150 times more than the human genome. GM presents immune-modulating, absorptive and metabolic functions (1). Antibiotics first and more recently probiotics—alive microorganisms beneficially affecting human health—represent the most significant agents capable of modulating GM composition (1). In current clinical practice, single and multi-strain preparations of probiotics, are used in gastrointestinal and non-gastrointestinal diseases with curative significant and promising results. In particular, the multistrain VSL#3® is often used for the treatment and maintenance period of ulcerative colitis (2).

The gut-liver axis consists of the interaction between GM, liver metabolism, and immune system; disruptions of this axis have been proved to be among the pathophysiological mechanisms associated with the onset and development of non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), until liver cirrhosis and cancer development (HCC) (3). Functional studies have shown how altered GM can dysregulate the opening of inter-enterocytes tight-junctions (TJ), responsible for the maintenance of the adequate gut permeability to antigens, toxic substances from intestinal lumen until bloodstream and liver are reached (3). Antibiotics and probiotics can regulate the gut-liver axis and have beneficial effects on NAFLD, NASH, liver cirrhosis, and HCC presentation and prevention (3). To date, there is only scarce evidence on the efficacy of certain probiotics on liver fat deposition, down-regulation of the inflammatory cascade, improvement of metabolic parameters in animal models (*Table 1*), and remission of symptoms in patients with ulcerative colitis (2).

Several animal models have shown that VSL#3 protects against NASH both in chemically-induced (dextran sulfate sodium-treated *ApoE–/–* mice) and diet-related (high fat diet-fed young rats and genetic *ob/ob* mice) setups (4). Moreover, in a particular diet-related NASH model, namely methionine-choline-deficient (MCD) diet-induced, VSL#3 failed to prevent both liver steatosis and inflammation although improved hepatic fibrosis (5). This finding is of great interest if compared to the possible impact on humans' health. In fact, the ketogenic high-fat diet is often used for brief periods to lose weight in particular categories as obese and bodybuilders and resulting in improved insulin sensitivity (1).

In light of these proven effects of VSL#3, the study of Jena *et al.* is of particular importance for the investigation of the potential effects of this multistrain probiotic preparation on several pathways of NASH development and carcinogenesis. The study employed a specific human-like animal model, farnesoid X receptor (FXR) knockout (KO) mice, which presented elevated BAs and developed steatosis and NASH, progressing spontaneously to liver cancer (4). These findings are consistent with previous works showing that inhibition of intestinal FXR signaling reduces obesity, insulin resistance, and fatty liver disease by modulation

Table 1 Main studies on animal models of NAFLD/NASH via gut microbiota modulation

Author	Animal model	Effects on liver via gut microbiota modulation
Rahman *et al*., 2016	F11 receptor gene knockout mice	NASH features, "inflammatory microbial taxa"
Pierantonelli *et al*., 2017	*Nlrp3* knockout mice	Abundance of Gram-negative species and translocation of bacteria were reduced after antibiotics, repairing liver and adipose tissue damages
Llorente *et al*., 2017	Sublytic *Atp4aSl*/*Sl* mice treated with proton pump inhibitors	Gut microbiota derangement and progression of liver disease
Sheng *et al*., 2017	WD-fed FXR KO male mice	Most serious NASH, greatest potential to develop HCC
Gart *et al*., 2018	Leiden mice	Gut microbiota changes and plasmatic/intestinal fatty acids concentration
Chen *et al*., 2019	*SIRT3* knockout HFD fed mice	Impairment of dysbiosis after HFD, ↑LPS levels and dysfunction, ↑ cannabinoid receptor 1 and 2 expressions in the colon and liver
de Sant'Ana *et al*., 2019	Caspases *1/11* and *Nlrp3* knockout HFD fed mice	↑ Proteobacteria and Firmicutes/Bacteroidetes ratio
Sun *et al*., 2019	Hamsters	Microbial modulation of bile acids metabolism, improvement of obesity-induced metabolic disorders
Schneider *et al*., 2019	Methionine-choline deficient diet in rats with induced NASH	↓ Gut microbiota diversity (different from human NASH patients)
Petrov *et al*., 2019	Germ-free, high-fat diet non-responder, Quercetin-supplemented HFD fed mice	↑ Stimulation of hepatic bile acid transporters, ↓ hepatic lipogenic and bile acid synthesis genes
Ahmad *et al*., 2020	C57BL/6J HFD fed mice	HFD induced gut microbiota changes
Cavallari *et al*., 2020	*NOD2* knockout mice	Gut microbiota changes, NAFLD development *NOD2*-dependent protection
Zhang *et al*., 2020	C57BL/6 high-fat and high-cholesterol diet male mice	Gut microbiota derangement

HFD, high-fat diet; LPS, lipopolysaccharide; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; *Nlrp3*, NLR family pyrin domain containing 3; *NOD2*, nucleotide-binding oligomerization domain-containing protein 2; *SIRT3*, NAD-dependent deacetylase sirtuin-3.

of hepatic and gut bacteria-mediated BA metabolism, and intestinal ceramide synthesis (6), while reduced expression of FXR has been detected in patients with liver cirrhosis and HCC (3). As BAs are produced by the conversion of hepatic cholesterol through hepatic and gut microbial enzymes, both nutrients and/or GM can affect metabolism and inflammation.

Sheng *et al*. chose this experimental animal model also according to previous data from their group showing that WD-fed FXR KO male mice have the most serious NASH and the greatest potential to develop HCC, as compared to WD-fed wildtype (WT) mice or healthy diet-fed FXR KO mice, irrespective of sex (7). VSL#3 was very effective in preventing hepatic lymphocyte infiltration and reduced hepatic cholesterol and triglycerides content. More interestingly, VSL#3 administration normalized expression of BA homeostasis genes, increased G-protein coupled BA receptor 1 (GPBAR1)-regulated signaling. These anti-inflammatory and anti-carcinogenic effects were consensual to GM reshuffling.

This study adds new pieces of evidence to the puzzle of microbial maps linked to NASH development/regression. Moreover, this investigation is in favor of using probiotics in the field of cancer prevention, a strategy acquiring growing evidence according to several positive findings in HCC from different causes as hepatitis B and C chronic infection, alcohol-related liver cirrhosis, and autoimmune liver diseases (8).

However, this study presents some limitations. It did not investigate the effect of a multistrain probiotic in

simple liver steatosis. Furthermore, little is known on the impact of single-strain probiotic belonging to VSL#3 on NASH development and single hepatic pathophysiological pathways. Neither strain-specific anti-carcinogenic effects have been investigated. Four months of VSL#3 supplementation are effective in NASH prevention in this mice model. However, these findings must be verified in human NASH patients. In this case, several issues have to be addressed, such as what single-strain or multistrain is likely to be more or at least as effective as in animal models or what dosage and for how long to treat patients, what is the impact of different dietetic regimens and lifestyles on the outcome of the treatment with VSL#3. In fact, the length of the protocol adopted in this paper raises the question about considering probiotics as an add-on treatment or as a long-term main-stay in lower inflammation and anti-cancerogenic state maintenance such as in IBD.

All these issues may seem drawbacks in the use of probiotics for NASH and HCC prevention in humans, while this and other incoming studies are unraveling the strain-specific, dose-finding, and duration of probiotics use in NAFLD to NASH and liver cirrhosis progression in humans. This could have long-term implications in HCC and liver cirrhosis prevention although dramatic diabetes and obesity epidemics are coming in this increasingly Westernized world.

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Footnote

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