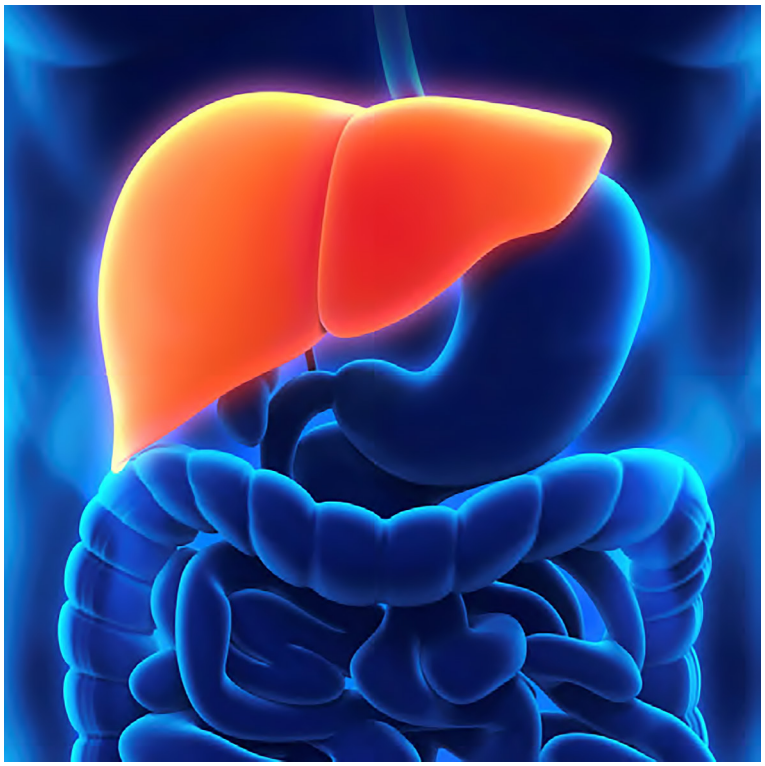
De Simone Formulation and Liver Diseases

Monograph

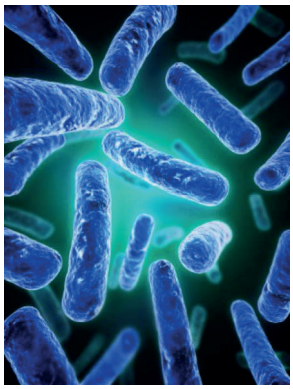


ONLY FOR HEALTHCARE PROFESSIONALS INFORMATION

CONCISE MONOGRAPH SERIES

1. Probiotics

Probiotics are live microorganisms that, when administered in an adequate amount, produce a beneficial effect to the host. The main properties of probiotics to explain their potential beneficial effects are changes in intestinal microbiota, improvements in the intestinal barrier, and modulation of the inflammatory response. Because probiotics are an “ecologic”, non-pharmacological and relatively cheap alternative to “classical” drugs, there has been growing interest in recent years regarding the possible usefulness of these therapeutical options in many fields of medicine.

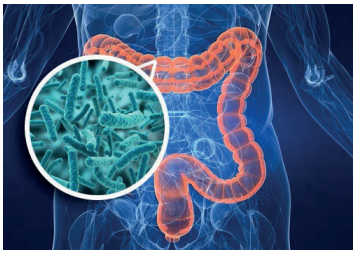


For decades, however, the implementation of probiotics in daily clinical practice has been limited.

The reasons for this restricted use include the high variety of probiotics with different properties and different quality, the shortage of high-quality trials, clinicians’ lack of confidence in these treatments, and the regulations that differ from those for drugs. Nevertheless, this scenario has been changing in recent years thanks to the recognition of concrete properties of several specific probiotics, the development of welldesigned clinical trials following the same strict guidelines used for drugs research, and the publication of results from these trials in high-quality journals. Moreover, the alarming increase in bacterial resistance as a result of the widespread use of antibiotics has created an urgent need for effective alternatives when modulation of intestinal microbiota is required.

2. Pathophysiology of liver diseases

Finally, pathological bacterial translocation can also produce a systemic inflammatory response that will contribute to the immune and hemodynamic alterations involved in the development of complications of cirrhosis: hepatic encephalopathy, infections, ascites, hepatorenal syndrome, variceal bleeding or acute-on-chronic liver failure (ACLF). In the case of hepatic encephalopathy, excessive production and absorption of ammonia in the gut plays a synergistic role with cerebral inflammation in the development of this complication.



Probiotics can therefore be useful as part of a global therapeutical approach of several liver diseases and in the prevention of the complications of cirrhosis by modulating gut microbiota, improving the intestinal barrier, and modulating immune alterations and inflammatory response.

3. The De Simone Formulation

The De Simone Formulation is a specific multispecies probiotic combination that consists of a mixture of 8 strains of bacteria: Streptococcus thermophilus DSM24731, Bifidobacterium breve DSM24732, Bifidobacterium longum DSM24736, Bifidobacterium infantis DSM24737, Lactobacillus paracasei DSM24733, Lactobacillus acidophilus DSM24735, Lactobacillus delbrueckii spp. bulgaricus DSM24734 and Lactobacillus plantarum DSM24730. The reason for the use of multispecies probiotics rather than a single strain is that these combinations can produce a more marked effect because of their potentially synergistic or additive effects on several steps of the pathway that we try to modify. We will review the most representative experimental and clinical studies that have evaluated this probiotic combination to date in liver diseases.

4. Experimental studies

Most experimental studies evaluating the De Simone Formulation in liver diseases have been conducted in models of NAFLD. In these models, the probiotic mixture has been reported to increase hepatic peroxisome proliferator-activated receptor (PPAR)-α expression and to decrease tumor necrosis factor TNFα levels and the activity of nuclear factor (NF)-kB, Jun N-terminal kinase (JNK), metalloproteinase (MMP)-2 and MMP-9 in the liver. Moreover, it has been shown that this probiotic mix decreases liver inducible nitric oxide synthase (iNOS) and cyclooxigenase (COX)-2 expression, and improves hepatic natural killer T cell (NKT) depletion. All these pathways are involved in the pathophysiology of NAFLD and, as a result of these effects, this probiotic mix has shown to decrease insulin resistance, steatosis, liver inflammation and fibrosis in several studies involving experimental models of NAFLD.

Chang *et al.* have evaluated the probiotic mix in a rat model of alcoholic intestinal injury and observed an increase in the intestinal expression of occludin and zonula occludens (ZO)-1 and a decrease in endotoxemia and serum TNFα. These results suggest a protective effect of this probiotic on the intestinal barrier leading to a decrease in bacterial translocation and systemic inflammatory response.

Regarding experimental cirrhosis, Sánchez *et al.* showed in a model of carbon tetrachloride (CCl_4)-induced cirrhosis in rats 4 that the probiotic combination decreased ascites formation, bacterial translocation and serum TNFα levels. Interestingly, these effects were associated with an increase in the intestinal expression of occludin and a decrease in ileal oxidative damage evaluated by malondialdehyde (MDA) levels. No significant changes were observed in gut microbiota evaluated by microbiological cultures. Therefore, these data suggest a main contribution of the improvement in intestinal barrier to explain the effects of this specific bacterial blend.

Rashid *et al.* recently evaluated the probiotic mix in another experimental model of portal hypertension: rats with common bile duct ligation. The authors observed that it prevented endothelial dysfunction in the mesenteric artery and the release of proinflammatory cytokines to systemic circulation.

Interestingly, in an experimental model of hepatocarcinogenesis in rats, Zhang *et al.* showed that the administration of the probiotic combination attenuated enteric dysbacteriosis, ameliorated gut inflammation and, more importantly, decreased the development of liver tumors. In a recent study in mice with common bile duct ligation, D'Mello *et al.* found that the probiotic mix decreased systemic inflammation, cerebral microglial activation and monocyte infiltration, and attenuated sickness behaviour. Further, Dr. Cudalbu very recently presented the data on a rat model of chronic liver disease induced hepatic encephalopathy.

Bile duct ligated rats were measured in vivo and longitudinally (before ligation and every 2 weeks after ligation for 8 weeks) using 1H Magnetic Resonance Spectroscopy, blood measurements and behavioural tests (Open Field). Probiotic supplementation was associated with lower increase in brain glutamine (typical feature of hepatic encephalopathy/ammonia detoxification in the brain), better brain osmoregulation band better performance in behavioural tests. Bifidobacteria was also measured longitudinally in rat feces and showed an increase (non-significant). This increase was negatively correlated with the increase of brain glutamine suggesting a positive treatment effect.

5. Clinical studies

5.1 Hepatic encephalopathy

Hepatic encephalopathy (HE) is the field of hepatology with most evidence of the usefulness of this specific combination of strains. Randomized studies including large number of patients have evaluated this probiotic in different settings: in minimal HE (MHE), in the prevention of HE recurrence, and in primary prophylaxis of HE. The most relevant trials are summarized in Table 1. A meta-analysis conducted by Saab *et al.* in 2015 analyzed 14 studies, 5 of which with the specific combination of strains confirming that the use of probiotics was effective in decreasing hospitalization rates, improving MHE and preventing progression to overt HE in patients with underlying MHE, with results similar to those with lactulose.

Minimal HE is a subtle cognitive dysfunction that can only be diagnsed using psychometric or neurophysiological tests.

Although minimal HE represents the mildest degree of HE, it is not devoid of clinical significance because it predisposes to overt HE, traffic accidents and falls, and it is associated to poor prognosis and deterioration of health-related quality of life. Mittal *et al.* performed a randomized study in 160 patients with cirrhosis and minimal HE, distributed into four groups. One group received the probiotic mix for 3 months, the second group was treated with lactulose, the third received L-ornithine L-aspartate (LOLA), and the fourth was a control group. The authors observed similar efficacy with the three treatments in terms of decrease in ammonia and improvement in psychometric tests and health-related quality of life compared to the control group. Minimal HE resolved at the end of study period in 35% of patients from the probiotic group, in 47.5% in the lactulose group, in 35% in the LOLA group, but only in 10% in the control group ($p=0.006$). A recent study by Pratap Mouli *et al.* confirmed a similar efficacy of probiotic mix and lactulose for 2 months in the improvement of minimal HE (69.7% vs 62.5%).

In another trial, Dhiman *et al.* also focused on the prevention of HE recurrence. The study was double-blind and the authors randomized 130 patients with cirrhosis and previous HE to receive the probiotic mixture or placebo for 6 months. Patients receiving the probiotic were less likely to need hospitalization due to HE during follow-up than patients in the placebo group (19.7% vs 42.2%, $p=0.03$) and they showed a statistically significant improvement in liver function that was not observed in the placebo group.

Finally, Lunia *et al.* aimed to evaluate the product in the setting of primary prophylaxis of HE. One hundred and sixty patients with cirrhosis and no previous HE were randomized either to a group supplemented with the probiotic mix for a mean period of 38.6 weeks or to a control group. The incidence of the first HE episode was significantly lower in the probiotic group (8.8%) than in the control group (20.3%). In addition, the authors observed a decrease in ammonemia and small intestinal bacterial overgrowth (SIBO) and an improvement in psychometric tests in the probiotic group.

Regarding safety, none of these studies reported relevant side effects attributable to the probiotic combination.

Ref.	N° and type of patients	Study agents	Duration	Results
Mittal 2011	160 Minimal HE	DSF vs lactuose vs LOLA vs control	3 months	↓ ammonia, improvement in psychometric tests and quality of life in the 3 treatment groups vs control
Agrawal 2012	235 Previous HE	DSF vs lactulose vs control	12 months	↓ HE recurrence in probiotics and lactulose vs control
Dhiman 2014	130 Previous HE	DSF vs placebo	6 months	↓ hospitalization for HE, improvement in psychometric tests, quality of life, liver function and inflammatory response
Lunia 2014	160 No previous HE	DSF vs control	mean 38.6 weeks	↓ HE incidence, ↓ ammonia, ↓ SIBO[1], improvement in psychometric tests
Mouli 2015	120 Minimal HE	DSF vs lactulose	2 months	Similar improvement in psychometric tests

Table 1. Randomized clinical trials evaluating the De Simone Formulation (DSF) in patients with cirrhosis and hepatic encephalopathy.

5.2 Portal hypertension

Considering the role of bacterial translocation and the proinflammatory state in the pathophysiology of hemodynamic alterations in cirrhosis, another potential target for probiotics is to decrease portal pressure to prevent complications, mainly variceal bleeding, ascites and hepatorenal syndrome.

Several authors have investigated the effect of the probiotic combination on portal pressure in patients with cirrhosis. Rincón *et al.* recently performed a non-comparative study including 12 patients with cirrhosis and ascites supplemented with the probiotic mix for 6 weeks. The authors observed a statistically significant decrease in the hepatic venous pressure gradient (HVPG, $P<0.001$), decrease in cardiac index and heart rate (both $P<0.01$) and an improvement in systemic hemodynamics (systemic vascular resistance $P<0.05$ and mean arterial pressure $P=0.06$) and increase in serum sodium in most patients ($P<0.01$).

Tandon *et al.* evaluated 8 predominantly compensated patients with cirrhosis and hepatic venous pressure gradient >10 mmHg supplemented with the probiotic combination for 2 months and they did not observe significant changes in portal pressure. In a non-controlled study, Loguercio *et al.* analyzed the effect of the probiotic mix for 3 months in several groups of patients with various liver diseases, including NAFLD. Patients with NAFLD showed a decrease in serum aminotransferases, oxidative damage and nitric oxide production. On continuation, the same group performed a double-blind placebo-controlled study that included 7 patients with decompensated cirrhosis and hepatic venous pressure gradient >10 mmHg supplemented with probiotic for 2 months and 8 supplemented with placebo. The mean change in hepatic venous pressure gradient was -11.6% in the probiotic group and +2.8% in the placebo group, but this difference did not achieve statistical significance, probably due to the small sample size.

Gupta *et al.* included 94 cirrhotic patients with large esophageal varices without previous variceal bleeding in a double-blind placebo controlled study. Patients were randomized to receive the probiotic mix, norfloxacin or placebo for 2 months. All patients received the standard prophylaxis of variceal bleeding with propranolol. The percentage of patients showing a hemodynamic response according to the change in hepatic venous

pressure gradient was higher in patients receiving probiotics (58%) or norfloxacin (54%) than in those receiving placebo (31%) (P=0.046). Serum TNFα decreased in the two first groups but not in the placebo group.

5.3 Obesity and non-alcoholic fatty liver disease (NAFLD)

In a non-controlled study, Loguercio *et al.* analyzed the effect of the probiotic mix for 3 months in several groups of patients with various liver diseases, including NAFLD. Patients with NAFLD showed a decrease in serum aminotransferases, oxidative damage and nitric oxide production.

Recently, Alisi *et al.* reported a double-blind placebo-controlled randomized trial in which they studied 44 obese children with NAFLD to evaluate the effect of the probiotic mix or placebo for 4 months. Although liver biopsy was not performed at the end of the study period, in children receiving the probiotic, the authors observed a statistically significant decrease in body mass index and an improvement in the severity of NAFLD evaluated by ultrasonography.

Obesity often leads to serious cardiovascular diseases and diabetes, and represents a heavy economic burden.

Rajkumar *et al.* conducted a randomized placebo-controlled study in overweight adults in 4 arms receiving the probiotic mix with or without Omega-3, Omega-3 alone or placebo and observed that the patients receiving the probiotics had significant reduction in total cholesterol, triglyceride, LDL (low density lipoprotein) and VLDL (very low density lipoprotein) and increase in HDL (high density lipoprotein) cholesterol. The combination with Omega-3 had a more pronounced effect on HDL, insulin sensitivy and amelioration of inflammation (hsCrP).

• Prevention of the first episode of hepatic encephalopathy
• Prevention of recurrence of hepatic encephalopathy
• Improvement in psychometric tests in patients with minimal hepatic encaphalopathy
• Decrease in the need for hospitalization due to hepatic encephalopathy
• Improvement in liver function tests
• Improvement in health-related quality of life
• Decrease in ammonemia
• Modulation of inflammatory response
• Decrease in portal pressure

Table 2. Summary of the clinical observations in patients with cirrhosis.

6. Conclusions

The specific mix of bacteria contained in the De Simone Formulation (DSF) has been mentioned for the first time in the Recommendations for probiotics 2015 from the proceedings of a workshop organized by Yale and Harvard Universities for the managament of liver conditions, in particular non alcoholic steatohepatitis and hepatic encephalopathy.

In particular level A evidence was recognized in hepatic encephalopathy.

Indeed, several randomized clinical trials including a large number of cirrhosis patients have demonstrated the possible application of the specific probiotic combination contained in the DSF in primary and secondary prevention of HE and in minimal HE. Other effects observed in these trials included an improvement in liver function tests and a decrease in the need for hospitalization. There is a potential role for DSF to decrease portal pressure and to prevent related complications in patients with cirrhosis. One main positive aspect represented by the use of DSF in these indications is that it improves the intestinal permeability, which prevents or reduces bacterial translocation thus reducing the inflammation of the liver. Other settings in which DSF could be useful include NAFLD and alcoholic liver disease. In particular, one important target may be obesity in children, which has become the most common cause of chronic liver disease in children

and is a significant burden on healthcare systems worldwide. The severity of hepatic steatosis is affected by intestinal permeability and intestinal bacterial overgrowth. There is a difference in the distinct composition of the gut microbiome among children and adolescents with nonalcoholic steatohepatitis (NASH), obese children without NASH, and healthy individuals, and modulation of the intestinal microbiota may offer an important therapeutic target for NAFLD as suggested by Miloh. Obesity in adults leads to high risks of metabolic syndrome and the use of this specific combination of probiotic strains to improve lipid profile, insulin sensitivity, and inflammatory responses may help reduce the risks of heart disease, diabetes, and stroke, in a healthy overweight population. The prevention of hepatocellular carcinoma, and the prophylaxis of bacterial infections avoiding the development of bacterial resistances observed with antibiotic prophylaxis are future targets for research.

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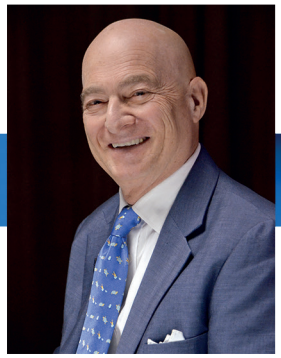

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La Lettre des probiotiques

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le blog du Prof. Claudio De Simone, pionnier du microbiote intestinal, inventeur du mélange probiotique (450 milliards de bactéries, 8 souches différentes)

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