Microbiota and Hepatic Encephalopathy: microbial dynamics and metabolism upon prebiotic, antibiotic and probiotic treatment

Andrea Mancini[1,2], Francesca Campagna[3], Piero Amodio[3], Cecilia Pravadelli[4], Kieran Tuohy[1]

[1] Nutrition and Nutrigenomic Group, Department of Food Quality and Nutrition, Research and Innovation Centre, Fondazione Edmund Mach (FEM), 38010 San Michele all'Adige (TN), Italy; [2]Department of Food Science, University of Parma, 43100 Parma, Italy; [3]Department of Medicine-DIMED, University of Padova, 35128 Padova, Italy; [4]Gastroenterology Unit, Santa Chiara Hospital, 38123 Trento, Italy.

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Introduction

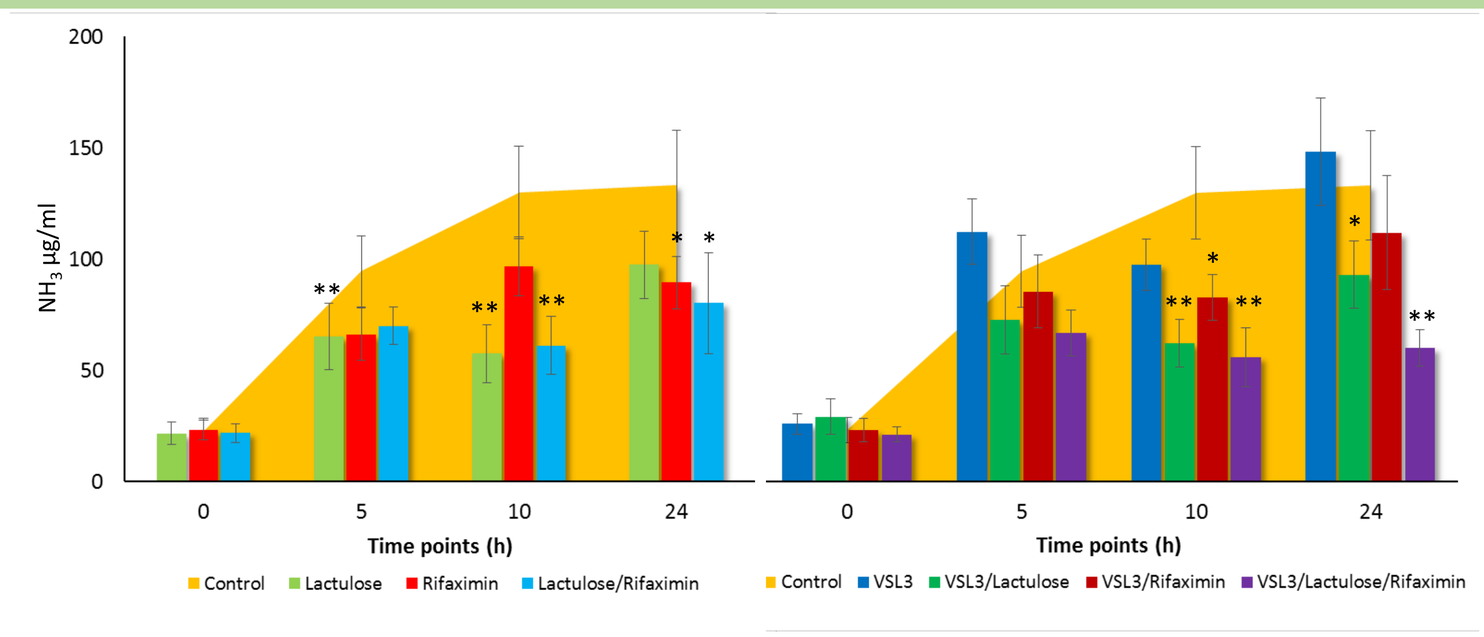
Gut ammonia production by microbial activity is one of the main factors implicated in Hepatic Encephalopathy (HE) [1, 2, 3]. Ammonia reducing strategies target mainly gut microbiota, by administration of prebiotics (Lactulose) [4], antibiotics (Rifaximin) [5, 6] and probiotics (VSL#3) [7]. Although the role of ammonia in the pathophysiology of HE is well established, the factors governing its productions by the gut microbiota are poorly understood.

Objectives

We investigated how gut microbiota modulation by prebiotics, antibiotics and probiotic treatments affected microbial ammonia production and the relative abundance of important members of the gut bacteria, within the cirrhotic environment.

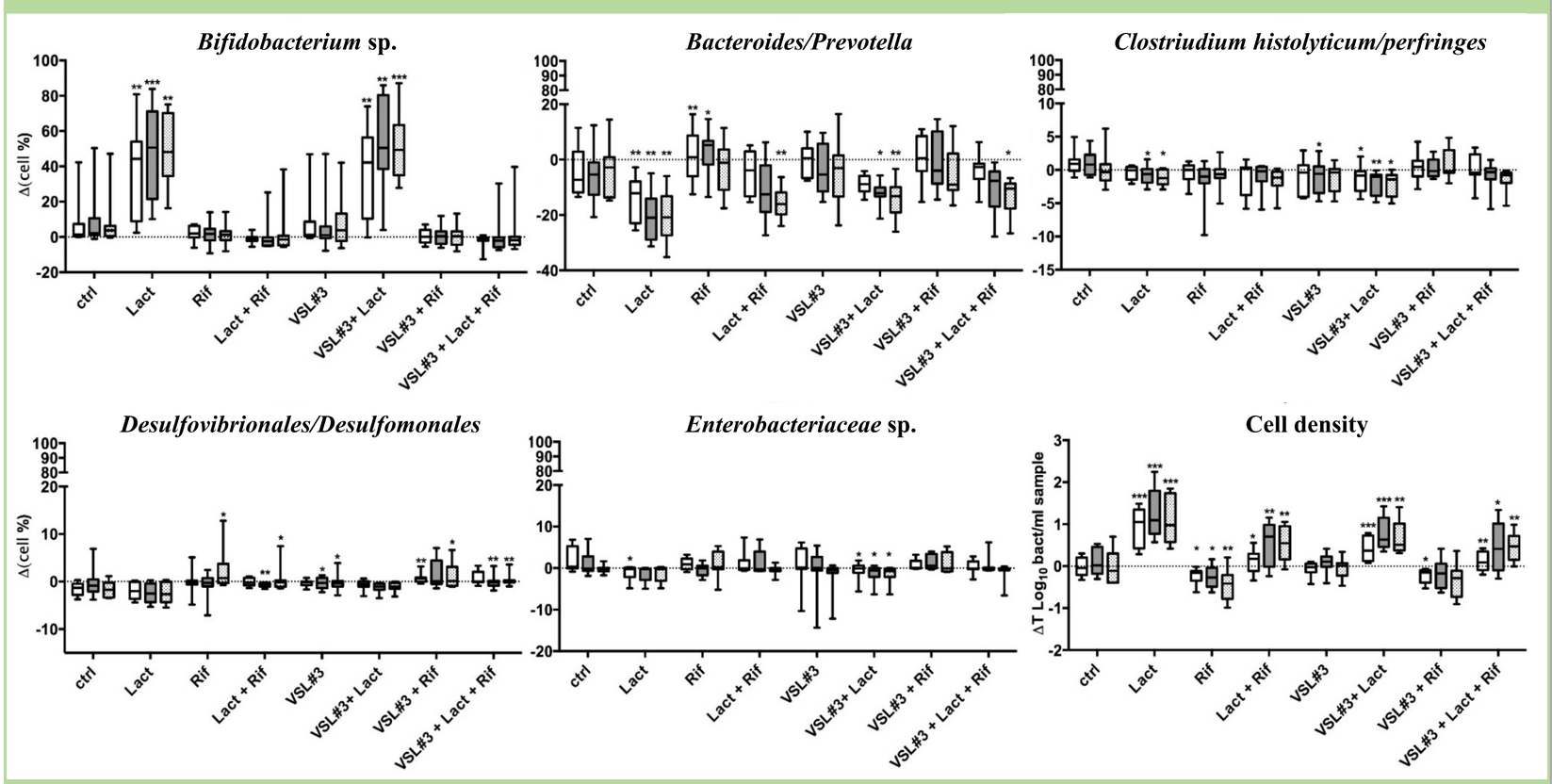
Results

1) Ammonia level assay



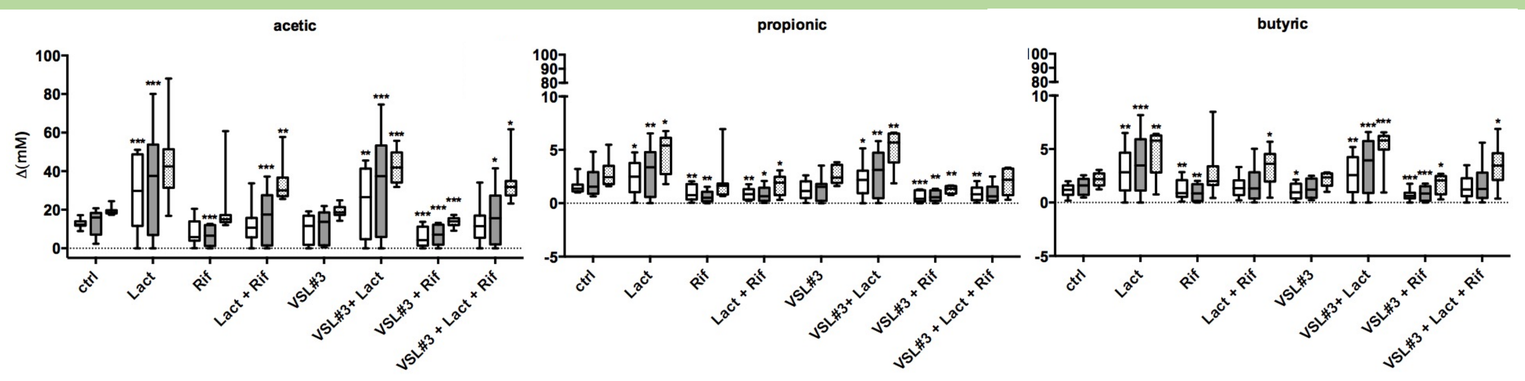
NH_3 concentration assessed by colorimetric method on the batch culture fermentation supernatant (mean ± sd, N = 10, in triplicates). *p-value≤0.05, **p-value ≤0.01, paired t-test, treatment vs ctrl.

2) ΔT bacterial enumeration



Average bacteria count differences, between times 5, 10, 24 and 0 for the considered FISH probes (mean ± sd, N = 10). *p-value≤0.05, **p-value ≤0.01, ***p-value ≤0.001, paired t-test, treatment vs ctrl. ΔT5; ΔT10; ΔT24
Ctrl, Control; Lact, Lactulose; Rifax, Rifaximin.

3) ΔT SFCA production



Average SFCA production differences, between times 5, 10, 24 and 0 for the considered FISH probes (mean ± sd, N = 10). *p-value≤0.05, **p-value ≤0.01, ***p-value ≤0.001, paired t-test, treatment vs ctrl. ΔT5; ΔT10; ΔT24
Ctrl, Control; Lact, Lactulose; Rifax, Rifaximin.

Take home messages

- NH_3 is reduced by all the treatments in a time dependent and combination manner, except for VSL#3 alone
- Over 24 hours NH_3 removal was retained although the efficacy of Lactulose was greater at T10
- VSL#3 NH_3 removal was augmented by the presence of prebiotic and antibiotic
- NH_3 reduction appears to be directly linked to increase in relative abundance in *Bifidobacteria*, better induced by prebiotic and antibiotic
- The Bifidogenic activity of Lactulose impact positively on SCFA production, with potential cross-feeding process for Priopionate and Butyrate

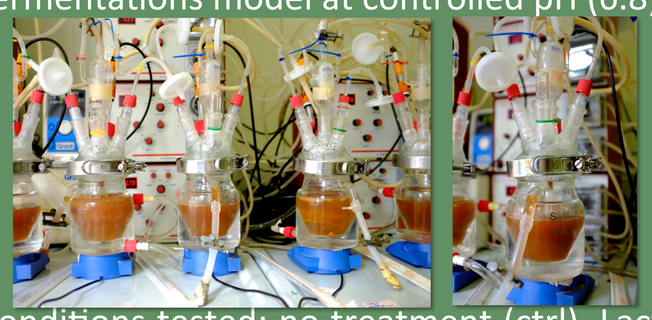
Conclusions

1. Gut microbiota modulation is a potential target for relieving the symptoms of HE by regulating colonic ammonia production;
2. In general lack of a direct correlation between microbial modification and NH_3 reduction for the tested treatments, suggest a modulation in ammonia production rather than increased size of the "colonic ammonia sink" via microbial biomass alone, as a possible mode of action.

The data pave the way to further investigations on gut metabolic activity and microbial cross-talk in the presence of Lactulose, Rifaximin and VSL#3.

Study design

1. Stool samples collection from ten cirrhotic patients (age 66±3.3; Child-Pugh A (n=9) and B (n=1); average MELD score 9±2.8) and three healthy subjects (age 63±2.5)
2. Sample inoculum in a 24-hour batch culture fermentations model at controlled pH (6.8)



Conditions tested: no treatment (ctrl), Lactulose (1%), Rifaximin (616µg/ml), Lactulose/Rifaximin, VSL#3 (initial [ ] of 10^8cell/ml), Lactulose/VSL#3, Rifaximin/VSL#3, Lactulose/Rifaximin/VSL#3

3. Microbial enumeration by Cytofluorimetric FISH (FCM-FISH) at 0, 4, 10 and 24 hours, using specific probes
4. NH_3 and SCFA were quantified at 0, 4, 10 and 24 hours

References

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[4] Sharma, P., Sharma, B.C. *Metab Brain Dis.* 2013 Jun;28(2):313-20. **[5]** Kavish, R., et al. *Metab Brain Dis*. 2013 Jun; 28(2): 307–312. **[6]** Bajaj, J.S. Aliment Pharmacol Ther. 2016 Jan;43 Suppl 1:11-26 **[7]** Sharma, B.C. and Singh, J. Metab Brain Dis. 2016 Apr 28.

Contact information:
andrea.mancini@fmach.it