Probiotic treatment improves outcome of chronic Hepatic Encephalopathy in BDL rats, an in vivo longitudinal 1H MRS study

Purpose: It is now accepted that intestinal dysbiosis is present in many diseases and plays an important role in their evolution/progression. Appropriate modulation of intestinal flora in patients with CLD may decrease intestinal ammonia production, reduce pro-inflammatory mechanisms in the gut thereby impacting intestinal permeability and consequent bacterial translocation. Therefore, our aim was to investigate the potential therapeutic effect of probiotic treatment with anti-inflammatory proprieties (VSL#3; actually available as Vivomixx® in EU and Visbiome in USA®) in CLD-induced HE. Methods: Probiotic treatment (VSL#3) was initiated 2 weeks before bile duct ligation (BDL) and was administrated daily by gavage (60-billion bacteria/kg rat) during the whole study. 38 adult Wistar male rats were divided into 4 groups: BDL-rats without treatment (‘BDL’, n=14), BDL-rats+VSL#3 (‘BDL+VSL#3’, n=14), sham-operated animals without treatment (‘sham’, n=5) and shams+VSL#3 (‘sham+VSL#3’, n=5). 1H-MRS in hippocampus was performed on a 9.4T-MR system using ultra-short-echo-time (TE=2ms) sequence at week0, 2, 4, 6 and 8, together with blood sampling to measure plasma bilirubin and NH4+. Open field test evaluated motor activity at week4, 6 and 8. Faeces were collected before treatment and every 2 weeks thereafter. Results and discussion: Increased plasma bilirubin confirmed the presence of CLD in both, BDL+VSL#3 and BDL-rats, with no difference between the two groups. However, important reduction of plasma NH4+ was observed in BDL+VSL#3 animals (Fig). Longitudinal in vivo 1H-MRS-measure of brain metabolites reinforced the positive findings about the effect of probiotic treatment. There was significantly smaller increase in brain Gln in BDL+VSL#3(+107%) compared to untreated BDL(+138%) at week8 (p=0.03). While the sum of osmolytes in BDL+VSL#3 was constant throughout the length of the study, the untreated BDL rats showed an incomplete osmoregulatory
response. Moreover, the increase of brain Gln in the BDL+VSL#3 group correlated negatively with their increase of genus Bifidobacteria in gut microbiota, suggesting a positive treatment effect. Differences among other brain metabolites are presented in the Table. With regards to behavioural tests: BDL+VSL#3 did not show the typical significant decrease in motor activity during the course of the disease, something we did observe in the BDL group. Interestingly, a subset (~30%) of treated rats did not display the characteristic increase in brain Gln and they had very stable performance in behavioural tests (all in CLD-condition proved by increased plasma bilirubin). Suggesting that in some individuals, VSL#3 may be highly protective. Conclusions: In BDL-rats, VSL#3 probiotic treatment was associated with positive effect on plasma NH4+, brain metabolite changes and motor activity, which may be related to increase Bifidobacteria in the gut. In a subset of animals, the effect seemed profound given that the characteristic neurometabolic profile with increased Gln was not observed. Taken together, these results are promising and warrant further investigation. The clinical implications of these findings are potentially far-reaching given that probiotics are generally safe and well-tolerated.

Table: Changes in brain metabolites expressed as %-change between week 0 (pre-BDL) and week 8 (post-BDL) and corresponding p-value for this change

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>BDL</th>
<th>BDL+VSL#3</th>
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</thead>
<tbody>
<tr>
<td>Glutamine</td>
<td>+138%, ****</td>
<td>+107%, **</td>
</tr>
<tr>
<td>myo-Inositol</td>
<td>-33%, ****</td>
<td>-24%, ***</td>
</tr>
<tr>
<td>Taurine</td>
<td>-8%, **</td>
<td>-13%, ****</td>
</tr>
<tr>
<td>Total Choline</td>
<td>-25%, ***</td>
<td>-20%, ns</td>
</tr>
<tr>
<td>Sum of osmolytes (Gln+mIns+Tau+tCho)</td>
<td>+9%, ***</td>
<td>0%, ns</td>
</tr>
</tbody>
</table>

Figure: Probiotic treatment improves Chronic Hepatic Encephalopathy in BDL rats
Probiotic treatment improves Chronic Hepatic Encephalopathy in BDL rats

Blood parameters

Plasma bilirubin showed significant increase in both BDL and BDL+VL5#3 group, with no difference between groups

Gut microbiota

However, BDL+VL5#3 displayed important decrease of plasma ammonium 6 weeks after BDL, observed already from week 4

Increase of bifidobacteria in gut microbiota in BDL+VL5#3 group (week -2 = before treatment)

Brain metabolites measured by 1H MRS

Longitudinal increase in brain glutamine in BDL and BDL+VL5#3

Gln was significantly lower in BDL+VL5#3

BDL+VL5#3 were able to keep constant sum of brain osomoloty (Gln=mino+Tau=tCho), compared to increase in BDL

Longitudinal evolution of main brain osomolotys (Gln, mino, Tau and tCho) in BDL and BDL+VL5#3

Behavioural tests

BDL+VL5#3 did not show significant decrease in motor activity, in contrary to BDL group

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