Effect of Rifaximin on gut microbiota in experimental liver fibrosis

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ABSTRACT

The aim of WP6 was to analyze the effect of Rifaximin on the gut microbiota of mice with liver fibrosis.

This study investigated the effect of Rifaximin *in vivo* and *in vitro*. Eight mouse groups of liver fibrosis were established and treated with or without Rifaximin in the drinking water. Faeces samples of all mice were collected before and after Rifaximin treatment and Colon and Cecum samples at the end of the treatment. Results of the microbiome analysis show, that Rifaximin has no microbiota interacting effect in liver fibrosis or in healthy control mice, since the alpha and beta diversity did not change after Rifaximin treatment and also not the relative abundance of bacteria in the gut microbiome.

METHODS

Generation of liver fibrosis in mice

The study included chow-fed control C_{57}Bl6/J mice (control) and 7 mice groups that were challenged with liver disease.

The first group received drinking water supplemented with 16% (v/v) Ethanol (EtOH), the second group was intoxicated by carbon tetrachloride (CCl_4), the third group was intoxicated by CCl_4 inhalation and with EtOH (CCl_4 + EtOH). The fourth group received a methionine choline deficient diet (MCD) and the fifth group received a methionine choline deficient diet (MCD) with EtOH (MCD + EtOH). The sixth group was fed with high fat western diet (WD) and the seventh group received western diet (WD) and EtOH (WD + EtOH). The animal size was 10 mice per group. Each of the experimental mouse groups was further divided into one group with additional rifaximin in the drinking water and one group without rifaximin in the drinking water for the treatment period of 7 weeks (Figure 1). Stool samples of all mouse groups were collected before and after the treatment, and cecum and colon samples at sacrifice.

Bioinformatics analysis

For analysis of the 16S rRNA (V4) amplicon sequence data an adjusted dada2 pipeline was used to process the sequencing data into an ASV (amplicon sequence variant) abundance table (Callahan BJ et al., 2016. Nat Methods 13:581–583). In a first step, primer sequences and ambiguous bases, as well as reads with shorter or longer than expected sequences were removed from the raw data using cutadapt. In an additional step, reads were trimmed at the 3 prime end based on sample-specific quality scores. The remaining reads were dereplicated into unique sequences and denoised separately for forward and reverse reads for each sample. Denoised forward and reverse reads were merged and read pairs without sufficient overlap or with any mismatch in the overlap region were removed. Finally, suspected chimeras were removed from the generated abundance table by internal abundance and sequence comparisons. The default taxonomic assignment of the detected ASVs was done using a naive Bayesian classifier algorithm comparing the ASV sequences to the SILVA reference database.

An abundance filter was addad and only ASVs with a relative abundance of at least 0.5% in at least one of the samples were kept. This reduced the total number of ASVs found in the 621 samples. Next, the taxonomic assignment was improved. In-silico extracted amplicons using the 515F/806R primers were used in the study from current versions of four reference databases (SILVA (https://www.arb-silva.de/documentation/release-138/), the genome taxonomy database (GTDB, https://gtdb.ecogenomic.org/), the ribosomal database project RDP (http://rdp.cme.msu.edu/), and the Unified Human Gastrointestinal Genome catalog (UHGG, https://www.ebi.ac.uk/metagenomics/genomes). For each ASV, reference amplicons matching perfectly to the ASV or the amplicons with the highest sequence identity to the ASV were used to manually improve the assignment of the ASVs. Finally, the ASVs were assigned to species level, to genus level, and to family level.

RESULTS

The body weight of mice which received CCl_4 and EtOH was significantly reduced compared to the control group. Mice which received MCD diet with and without EtOH lost the most weight. After WD treatment mice had an increased body weight (Figure 2). The taxonomic overview of the bioinformatics analyses show a strong effect of the MCD treatment in the stool samples. Samples after the treatment with MCD diet show higher proportions of *Verrucomicrobiae*, *Desulfovibrionia*, and *Bacilli* and lower proportions of *Bacteroidia* than other samples. Samples of animals that received EtOH had higher relative abundances of *Actinobacteria*, except for MCD treated animals (Figure 3). Additionally, WD and MCD fed mice show a reduced alpha diversity compared to control samples. EtOH treated mice show a mildly reduced alpha diversity in their cecum samples compared to control animals (Figure 4). The beta diversity shows a shift of MCD diet vs. WD and normal chow (Figure 5).

However, all treatment groups differed significantly in the overall microbiome composition from control group after the treatment period. Taken together, Rifaximin treatment had no effect on the alpha, nor on the beta diversity or on the phylum to phylum ratios in the experimental mouse groups.

CONCLUSION

The microbiome composition was changed significantly in all liver fibrosis groups compared to control mice. After 7 weeks of MCD diet the alpha diversity was reduced and there was a clear shift of the beta diversity. Controversitly, WD induced an increased alpha diversity and EtOH increased the abundance of *Actinobacteria* in the gut microbiome.

However, Rifaximin was not able to effect the gut micobiome abundance significantly. Thus, the treatment of liver fibrosis with Rifaximin is not suitable to influence the gut microbiome after 7 weeks.

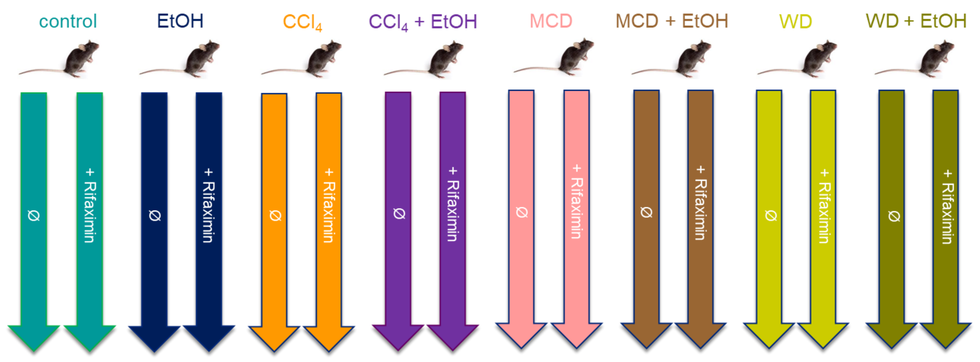

Figure 1: Experimental mouse groups to investigate the Rifaximin effect on the gut microbiota.

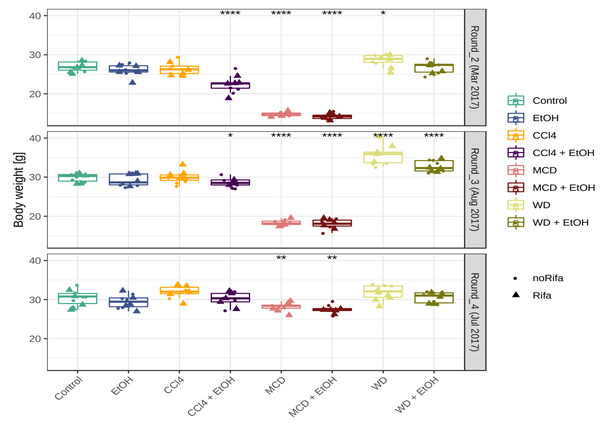

Figure 2: Body weight after 7 weeks of treatment in the different mouse models of liver fibrosis with and without additional Rifaximin treatment.

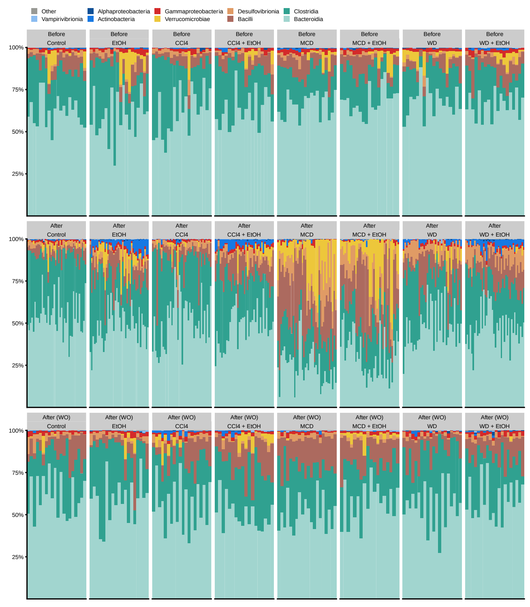

Figure 3: Taxonomic profiles of all samples at class level before and after the treatment period of 7 weeks.

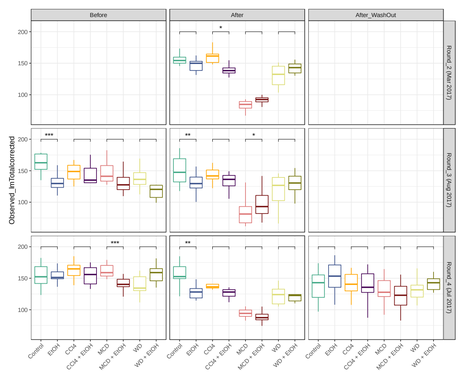

Figure 4: The alpha diversity of MCD diet treated mice resulted in a strong alpha diversity reduction. Furthermore, WD lowered alpha diversity but not as strongly as MCD. MCD diet together with EtOH reduced fecal richness after rifaximin treatment.

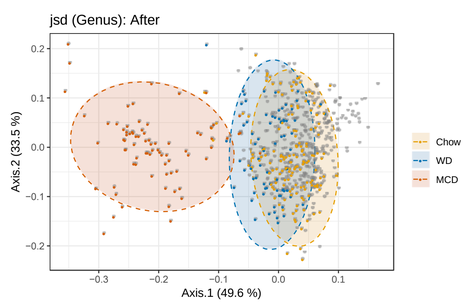

Figure 5: Beta diversity after WD and MCD diet in the gut microbiome.

All the authors are very grateful to Dikra Zouiten and Evelin Süß for their excellent technical assistance. The authors were supported by grants from the Deutsche Forschungsgemeinschaft (SFB TRR57 and CRC1382), European Union's Horizon 2020 Research and Innovation Programme (Galaxy, No. 668031, MICROB-PREDICT, No. 825694, and DECISION No. 84794), Societal Challenges-Health, Demographic Change, and Well-Being (No. 731875), Cellex Foundation (PREDICT), LIVERHOPE (No. 731875), Enable (funded by Hessian Ministry of Higher Education, Research, Science and the Arts) and deep-HCC (German Federal Ministry of Education and Research).

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 825694. This reflects only the authors' view and the European Commission is not responsible for any use that may be made of the information it contains.

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