Manipulating Microbiota in Liver Disease

Jasmohan S Bajaj, MD
Division of Gastroenterology, Hepatology and Nutrition, Virginia Commonwealth University and McGuire VA Medical Center, Richmond, Virginia, USA
Outline and Questions to be asked

• Why is it relevant to study microbial change in liver disease?
• What is lacking in the current microbial therapies for liver disease?
  • What are the levels of therapy in reducing the severity of liver disease?
• Is microbial therapy going to be enough?
Microbial Analysis and Outputs
Phylogenetic Tree of Life: Microbes, Fungi and Humans

- **Proteobacteria**
- **Gammaproteobacteria**
- **Enterobacterales**
- **Enterobacteriaceae**
- **Escherichia**
- **Escherichia coli**
Products of bacterial metabolism

- **Carbohydrates**: Short chain fatty acids
- **Phytochemicals**: Phenolic acids
- **Protein**: Phenolic acids, Ammonia, Polyamides
- **Fat**: Bile acids with taurine and secondary bile acids
- **Xenobiotics**: Carcinogens
- **Alcohol**: Acetaldehyde
Microbial Outputs

- Type of bacteria
- Presence/absence
- Richness: count of individual bacterial types
- Diversity: count of individual types and their abundance
- Relative abundance: percent present compared to the total abundance
Levels of study in microbiome research

• Which microbes are present in the GI tract? (culture-dependent or independent techniques)
  • Stool
    • GI tract mucosa
    • In peripheral systems
• What are GI tract microbes doing? (metatranscriptomics, metaproteomics, metabonomics/metabolomics).
• What microbial genes are present in the GI tract? (metagenomics)

Ultimately, the relationship with host state and outcomes is essential
Relevance of microbial change in liver disease

- Liver diseases, especially alcohol and NAFLD, initiate microbial change independent of liver injury
- This microbial change could worsen the liver injury phenotype
- Cirrhosis of the liver, the end-stage of fibrosis, can affect the microbiota
- Microbial change, including bacterial translocation, is associated with systemic and intestinal inflammation

**Biosensors: Keystone and Indicator organisms**

**Keystone organisms**: that has a disproportionately large effect on its environment relative to its abundance

*Adequate presence* = *Good health of the overall ecosystem*

**Indicator organisms**: that defines a trait of the environment which are also known as sentinel organisms, i.e. organisms which are ideal for biomonitoring

*E.g. Coliforms to monitor water quality*

**Ideal Biosensor: Combination of Keystone and Indicator Organisms**
The Cirrhosis Dysbiosis Ratio Parallels Cirrhosis Severity

Cirrhosis Dysbiosis ratio was also stable over time, worsened with the development of the first episode of hepatic encephalopathy and was worse in those who were subsequently hospitalized

Microbial change is linked to outcomes in cirrhosis

- Microbial change can predict inpatient and outpatient outcomes in cirrhotic patients
- Microbiota are associated with an altered gut-liver-brain axis resulting in \textit{Hepatic encephalopathy (HE)}

Cognitive function and brain inflammation and edema are related to microbes in cirrhosis= HE


Death in Cirrhotic Inpatients

Hospitalizations in Outpatients

The Hong Kong Association for the Study of Liver Diseases
Pathophysiology of HE

Bajaj JS Hepatology 2015, Dasarathy et al J Hepatol 2016
Gut microbiota are necessary for brain inflammation (microglial and glial) in cirrhotic mice

4 mouse groups: GF, GF made cirrhotic using CCL4 gavage, Conventional control and Conventional mice made cirrhotic using CCL4 gavage

Kang, Bajaj et al Hepatology 2016
Current microbial therapies need more precision

Pre-cirrhosis

• Probiotics: very poor evidence in NAFLD and alcoholic liver disease with multiple formulations used and for very small durations

Cirrhosis: Hepatic Encephalopathy

• Probiotics: Good data with VSL#3 for readmission prevention but not recurrence of hepatic encephalopathy
• Prebiotics/Laxatives: Poor evidence for lactulose and mechanistically likely to be a laxative
• Antibiotics: Rifaximin has the best evidence profile but it is expensive and also does not prevent all HE episodes

Levels of therapy in reducing the severity of liver disease

• Control etiology: Not likely microbial
• Affect inflammatory milieu locally and systemically: Oral cavity
• Change gut microbial composition and hopefully function: FMT, Proton pump inhibitors, Engineered bacteria and Dietary modification
• Change gut microbial interaction with other microbiota: Interaction with fungi
Systemic and Local Inflammatory Control
Oral Microbiota is different from stool microbiota compositionally in healthy controls and in cirrhotic patients

Periodontal therapy improves gut microbiota and endotoxemia

<table>
<thead>
<tr>
<th></th>
<th>Cirrhosis</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-therapy</td>
<td>Post-therapy</td>
</tr>
<tr>
<td>MELD score</td>
<td>9.5 ± 3.3</td>
<td>8.6 ± 2.7*</td>
</tr>
<tr>
<td>WBC count</td>
<td>6.11 ± 1.90</td>
<td>5.67 ± 1.85*</td>
</tr>
<tr>
<td>Endotoxin (EU/ml, median, IQR)</td>
<td>0.18(0.05)*</td>
<td>0.12(0.07)#</td>
</tr>
<tr>
<td>TNF (pg/ml, median, IQR)</td>
<td>17.3(9.1)*</td>
<td>15.1(8.3)#</td>
</tr>
</tbody>
</table>

Bajaj et al. Am J Physiol 2018
Oral-Gut-Hepatic Axis

Control the etiology
Prevent Decompensation
Liver Transplant

Periodontal Therapy
Avoid smoking
Avoid alcohol
Oral hygiene

Cirrhosis

Poor dentition + oral barrier dysfunction
Sensitization and further liver injury
SIBO + Intestinal barrier dysfunction + Bile acid dysregulation

Proteobacteria overabundance, impaired mucosal immunity

Systemic Endotoxemia

Bacterial translocation and increased inflammatory mediators: IL-1, IL-6, TNF

Intestinal Dysbiosis

Inflammation, vagal dysfunction, distal migration due to bile acid and gastric acid suppression

Non-absorbable Abx
Probiotics
Avoid unnecessary PPI

Acharya, Sahingur and Bajaj
JCI Insight 2018
Microbial Composition and Functional Change
Proton Pump Inhibitors Initiation and Withdrawal can Change Microbiota in Liver Disease

PPIs Exacerbate Murine Alcoholic Liver Injury Through Enterococcus

Microbiota changes
Yellow: PRE Red=POST

Adding PPIs Oralizes Stool Microbiota in Compensated Cirrhosis and Healthy Controls

Similar changes are found in Advanced Cirrhosis with Reversal after PPI Withdrawal

Manipulation of microbiota: Fecal Microbiota Transplant
Particular challenges of FMT in HE

- Generally a much more advanced population
- Prone to potentially life-threatening infections, including those that are initiated from the gut
- Avoiding antibiotics post-FMT may not be feasible
- Many are already on rifaximin and SBP prophylaxis
- Directed donor vs. universal donor?
Case Report of FMT in the management of hepatic encephalopathy

Kao et al. Hepatology 2015
Healthy patients → HE patients → Train HE Classifier to rank donors → OpenBiome donors

Material from one stool sample from the donor with highest Lachnospiraceae/Ruminococaceae used for the FMT-assigned group

RCT of FMT enema vs. standard of care with safety as primary endpoint

Outpatient cirrhosis and recurrent HE

Patients divided into standard of care or FMT group with 150 day follow-up

IN THE FMT GROUP COMPARED TO STANDARD OF CARE:
- Reduced hospitalizations
- Improved cognition
- Reduced HE episodes
- Recovery of antibiotic-associated collapse in microbial diversity

Bajaj et al
Hepatology 2017
Fecal Microbiota Transplant Changes Microbial Function

**Specific Microbes**
- Reduce Ammonia
- And improve survival
- In Murine models

**In Humans FMT improves cognition, restores SCFA and Bile Acid profile**

**SCFA**

**Bile Acids**

Shen et al JCI 2015, Kao et al Hepatol 2015
Bajaj et al Hepatol 2017, Bajaj et al Hepatol 2018
Mediterranean diet in cirrhosis = better diversity and lower hospitalizations compared to Western Diet

• Cohort of 296 Turkish and US-based controls, compensated and decompensated cirrhotic patients was included and followed for 90 days

• Turkish subjects had similar diversity due to greater consumption of fermented milk products.

• There was a significantly lower risk of 90 day hospitalization in Turkish compared to American cirrhotic patients

• On Cox and binary logistic regression, microbial diversity was protective against 90-day hospitalizations, along with coffee/tea, vegetable and cereal intake.

Bajaj JS, Idilman R et al, Hepatol 2018
Engineering Probiotic Bacteria to Consume Ammonia

Engineered probiotic bacteria have the potential beyond ammonia control in hepatic encephalopathy

Hepatic Encephalopathy Pathogenesis

Hepatic Inflammation
Intestinal permeability
Liver Fibrosis
Hepatocyte Necrosis / Apoptosis

Collagen I
Density Units
Healthy Disease model Probiotic in disease model

α-SMA
Density Units
Healthy Disease model Probiotic in disease model

Serum-endothelin (U/mL)

Healthy Control Vehicle SYN1020
Toxin-induced liver disease model

AST
ALT

Heat inactivated 1020 SYN1020
Heat inactivated 1020 SYN1020

Slide courtesy of Synlogic Pharmaceuticals
Microbial Interactions
Bacterial and fungal interactions are relevant in liver disease

Fungi Worsen Alcoholic Liver Injury In Murine models

Gut Fungi are Related to Gut Bacterial Diversity and Worsen with Antibiotic use, being replaced by Candida

Yang et al JCI 2017, Bajaj et al Gut 2017
However, are cirrhosis and chronic liver disease microbial diseases?

In other words without controlling the liver disease etiology, can we expect an improvement by just affecting the microbiota?
Liver cirrhosis requires liver injury: microbes potentiate but do not cause it independently

<table>
<thead>
<tr>
<th>Stool Donor</th>
<th>Systemic Inflammation</th>
<th>Liver Inflammation</th>
<th>Liver injury/cirrhosis</th>
<th>Bacterial translocation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy human</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Non-drinking Cirrhotic human</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Non-drinking Cirrhotic with HE</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Actively drinking non-cirrhotic human</td>
<td>+</td>
<td>+</td>
<td>Only if fed alcohol</td>
<td>Only if fed alcohol</td>
</tr>
<tr>
<td>Actively drinking cirrhotic human</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Conclusions

• Microbial changes are an integral part of the altered gut-liver axis in cirrhosis and pre-cirrhotic liver disease
• They are complicit but not necessarily causative of liver injury without the direct liver injury also
• Microbial treatment in liver disease has to be accompanied by treatment of the liver disease etiology
• Current therapies can be improved by precision changes in microbiota
• Specific means by which we can potentially improve outcomes
  • Regular dental cleaning and avoid periodontitis
  • Withdraw unnecessary PPI use
  • Consider the use of antibiotics that can encourage fungal infections
  • Focus on therapies related to bacterial function
  • Emphasis on fermented, probiotic foods
  • Make every effort to combine microbial therapy with etiological therapy as well
Acknowledgements

VCU Microbiology/Immunology
• Phillip Hylemon, PhD
• Huiping Zhou, PhD
• DJ Kang, MS
• Genta Kakiyama, PhD

VCU/VA Gastroenterology and Hepatology
• Arun J Sanyal, MD
• Douglas M Heuman, MD
• William M Pandak, MD
• Richard K Sterling, MD
• R Todd Stravitz, MD
• Velimir Luketic, MD
• Puneet Puri, MD
• Michael Fuchs, MD
• Muhammad S Siddiqui, MD
• Scott C Matherly, MD
• Binu John, MD
• Hannah Lee, MD

GMU Environmental Sciences
• Patrick M Gillevet, PhD
• Masoumeh Sikaroodi, PhD
• Naga Betrapally, MS
• Swati Dalmet, MS

VCU/VA coordination
• Melanie White, RN
• Edith Gavis, RN
• Jill Meador, RN
• Andrew Fagan, BS

Funding: VA Merit Review I0CX001076, RO1DK089713, RO1AA020203