The Total Western Diet and Vancomycin Increase Inflammation Mediated Colorectal Cancer

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Factors

Inflammation

Gut Microbiome

Diet & Lifestyle
Pre-clinical studies (i.e. Animal Models)

AOM/DSS

Antibiotics  <->  TWD
The Question

What is the effect of the total Western diet, vancomycin-induced changes to the gut microbiome, and the combination of the two on colorectal cancer in the presence of DSS-induced inflammation?
Hypothesis

• We hypothesize that *vancomycin* treatment will decrease the overall tumor burden, as measured by total tumor volume/colon, in mice fed the total Western diet in the presence of DSS-induced inflammation, and that this attenuation will be supported by a significant diet x treatment interaction.
Study Design

- A/VM
- A/Wa
- T/VM
- T/Wa

AOM/DSS

144 mice; 9 cages per group x 4 mice per cage (n=36)
Endpoints

- Tumor Burden (total tumor volume/colon)
- Tumor Multiplicity (number of tumors/colon)
- Tumor Size \((\text{mm}^3)\)
- Mucosal Injury and Inflammation
- Visual Colitis Assessment
- Microbiome
  - Taxonomic Summaries
  - Species Richness
  - Community Similarity
Statistical Analysis

• All data were analyzed using SAS On Demand.
• Data were tested for the main effects of diet, vancomycin treatment, and the diet x treatment interaction.
• Cage effect was taken into account when performing statistical analysis.
• Group mean analysis was performed using the Ryan-Einot-Gabriel-Welsh (REGWQ) test.
Results

Colitis Assessment 1 (1 day post-DSS)

Disease Activity Index

Diet:
- Water
- Vancomycin

AIN
- Water: a
- Vancomycin: b

TWD
- Water: bc
- Vancomycin: c

Statistical Tests:
- Diet: $P<0.01$
- AB: $P=0.01$
- D*AB: $P=0.22$
Results

Colitis Assessment 2 (14 days post-DSS)

- **Diet:** $P<0.01$
- **AB:** $P<0.01$
- **D*AB:** $P=0.31$

- **Water**
- **Vancomycin**

<table>
<thead>
<tr>
<th>Diet</th>
<th>Disease Activity Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIN</td>
<td>a</td>
</tr>
<tr>
<td>TWD</td>
<td>b</td>
</tr>
</tbody>
</table>

Legend:
- a
- ab
- b
- c
Results

Mucosal Injury (Recovery)

Diet: $P=0.01$
AB: $P=0.32$
D*AB: $P=0.81$
Results

Mucosal Injury (Terminal)

Diet: $P=0.64$
AB: $P=0.51$
D*AB: $P=0.04$
Results

Inflammation Score (Recovery)

Diet: $P<0.01$
AB: $P=0.60$
D*AB: $P=0.27$

Diet:
- AIN
- TWD

Groups:
- Water
- Vancomycin

Significance:
- a
- ab
- b
Results

Inflammation Score (Terminal)

Water
Vancomycin

Diet: $P<0.01$
AB: $P=0.13$
D*AB: $P<0.01$

<table>
<thead>
<tr>
<th>Diet</th>
<th>Inflammation Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIN</td>
<td>a</td>
</tr>
<tr>
<td>TWD</td>
<td>b</td>
</tr>
</tbody>
</table>

Notes:
- a vs. b: significant difference
- b vs. b: no significant difference

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Results

Taxonomic Summaries by Treatment: Phylum Level

- **Verrucomicrobia**
- **Firmicutes**
- **Actinobacteria**

**T/Wa**
- Verrucomicrobia: Light Gray
- Firmicutes: Light Blue
- Actinobacteria: Orange

**A/VM**
- Verrucomicrobia: Light Gray
- Firmicutes: Light Blue
- Proteobacteria: Pink

**T/VM**
- Verrucomicrobia: Light Gray
- Firmicutes: Light Blue
- Proteobacteria: Pink
## Results

### Taxa with largest differences

<table>
<thead>
<tr>
<th>Type</th>
<th>A/Wa</th>
<th>T/Wa</th>
<th>A/VM</th>
<th>T/VM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Verrucomicrobia</td>
<td>5.2%</td>
<td>1.9%</td>
<td>37.5%</td>
<td>37.9%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Firmicutes</td>
<td>81.4%</td>
<td>87.7%</td>
<td>29.8%</td>
<td>29.3%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Actinobacteria</td>
<td>11.2%</td>
<td>8.9%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Proteobacteria</td>
<td>0.1%</td>
<td>0.0%</td>
<td>32.5%</td>
<td>32.7%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Bacteroidetes</td>
<td>1.9%</td>
<td>1.3%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
Results

Taxonomic Summaries by Treatment: Genus Level

- Akkermansia
- Allobaculum
- Clostridales
- Lactococcus
- Bifidobacterium
- Enterobacteriaceae
- Sutterella

A/Wa
T/Wa
A/VM
T/VM
## Results

<table>
<thead>
<tr>
<th>Taxa with largest differences</th>
<th>A/Wa</th>
<th>T/Wa</th>
<th>A/VM</th>
<th>T/VM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>p_Verrucomicrobia: g_Akkermansia</td>
<td>5.1%</td>
<td>1.9%</td>
<td>37.6%</td>
<td>37.9%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>p_Firmicutes: g_Allobaculum</td>
<td>42.7%</td>
<td>52.7%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>p_Firmicutes: o_Clostridiales</td>
<td>7.7%</td>
<td>5.8%</td>
<td>0.9%</td>
<td>0.4%</td>
<td>0.05</td>
</tr>
<tr>
<td>p_Firmicutes: g_Lactococcus</td>
<td>23.1%</td>
<td>17.4%</td>
<td>27.3%</td>
<td>26.8%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>p_Actinobacteria: g_Bifidobacterium</td>
<td>10.6%</td>
<td>8.5%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>p_Proteobacteria: f_Enterobacteriaceae</td>
<td>0.0%</td>
<td>0.0%</td>
<td>15.3%</td>
<td>20.5%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>p_Proteobacteria: g_Sutterella</td>
<td>0.0%</td>
<td>0.0%</td>
<td>8.9%</td>
<td>5.7%</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
Results

![Observed Operational Taxonomic Units](chart.png)

- **Water**
- **Vancomycin**

**Diet**
- P = 0.48
- AB: P < 0.01
- D*AB: P = 0.47
Results

PD Whole Tree

\[
\log_{10}(\text{Index Score})
\]

<table>
<thead>
<tr>
<th>Diet</th>
<th>Water</th>
<th>Vancomycin</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIN</td>
<td></td>
<td>a</td>
</tr>
<tr>
<td>TWD</td>
<td>b</td>
<td>b</td>
</tr>
</tbody>
</table>

Diet: \( P=0.05 \)
AB: \( P<0.01 \)
D*AB: \( P=0.73 \)
Results

![Chao1 Index Graph]

- **Diet**: P = 0.48
- **AB**: P < 0.01
- **D*AB**: P = 0.70
Results
Results

![Tumor Size Graph]

- **Diet:** $P=0.22$
- **AB:** $P=0.08$
- **D*AB:** $P=0.39$
Results

**Tumor Multiplicity**

- **Water**
- **Vancomycin**

- **Diet:** $P<0.01$
- **AB:** $P<0.01$
- **D*AB:** $P=0.06$

The diagram shows the comparison of tumor multiplicities between water and vancomycin under different diets (AIN and TWD). The results indicate a significant difference in tumor multiplicities between the two groups.
Results

Tumor Burden

- **Water**
- **Vancomycin**

<table>
<thead>
<tr>
<th>Diet</th>
<th>(mm³)^(1/3)</th>
<th>AIN</th>
<th>TWD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>a</td>
<td>a</td>
<td></td>
</tr>
<tr>
<td>Vancomycin</td>
<td>b</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Diet: *P*<0.01  
AB: *P*<0.01  
D*AB: *P*=0.17
Summary

• TWD and VM increase DSS-induced colitis.
• TWD increases gut inflammation long-term.
• TWD increases colonic mucosal injury immediately following DSS treatment.
• VM alters gut microbial composition.
  • Relative taxonomic abundance
  • Species Richness
  • Community Similarity
• VM and TWD significantly increase colon tumorigenesis.
  • Tumor burden and multiplicity
Conclusion

The total Western diet and vancomycin-induced changes to the gut microbiome increase inflammation-induced colitis as measured by total tumor volume.