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  T/VM

A/Wa  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 (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: $P<0.01$
AB: $P=0.01$
D*AB: $P=0.22$
Results

Colitis Assessment 2 (14 days post-DSS)

Disease Activity Index

- Water
- Vancomycin

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

Diet: AIN, TWD

Bars labeled with different letters indicate significant differences.
Results

Mucosal Injury (Recovery)

- Water
- Vancomycin

Injury Score

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

Diet

<table>
<thead>
<tr>
<th>Diet</th>
<th>Injury Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIN</td>
<td>a</td>
</tr>
</tbody>
</table>
| TWD  | a            | a

Significance: a

12
Results

Mucosal Injury (Terminal)

- Water
- Vancomycin

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

Injury Score

Diet

- AIN
- TWD

*a*
Results

**Inflammation Score (Recovery)**

<table>
<thead>
<tr>
<th>Diet</th>
<th>Water</th>
<th>Vancomycin</th>
</tr>
</thead>
<tbody>
<tr>
<td>AI N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T W D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

Inflammation Score (Terminal)

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

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

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

Enterobacteriaceae
Sutterella
<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>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](image)

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

The figure illustrates the observed operational taxonomic units (OTUs) under different dietary conditions. The results indicate a statistically significant difference in OTU counts between the Water and Vancomycin treatments, with further significance noted in the interaction term $D^*AB$.
Results

PD Whole Tree

![Graph showing log₁₀(Index Score) for AIN and TWD diets with Water and Vancomycin treatments.](image)

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

Chao1 Index

Index Score

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

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

![Bar graph showing tumor size comparison between Water and Vancomycin diets.](image)

- **Tumor Size**
  - **Diet**:
    - AIN: Water
    - TWD: Vancomycin

- **Log$_{10}(1+\text{mm}^3)$**

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

![Bar chart showing tumor multiplicity for AIN and TWD diets with and without vancomycin.](chart)

- **Tumor Multiplicity**
- **Y-axis:** Number of tumors
- **X-axis:** Diet (AIN, TWD)
- **Legend:**
  - Water
  - Vancomycin

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

Tumor Burden

- Water
- Vancomycin

<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>a</td>
<td>b</td>
</tr>
</tbody>
</table>

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

$(\text{mm}^3)^{1/3}$
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.