Exploiting the Gastrointestinal Microbiota as a Therapeutic Target for Type 1 Diabetes

Bryn Davis  
*Clemson University*

Katrina Ross  
*Clemson University*

Halee Bryant  
*Clemson University*

Kelsey Tackeberry  
*Clemson University*

Caitlyn Blake-Hedges  
*Clemson University*

See next page for additional authors

Follow this and additional works at: https://tigerprints.clemson.edu/foci

Recommended Citation

Davis, Bryn; Ross, Katrina; Bryant, Halee; Tackeberry, Kelsey; Blake-Hedges, Caitlyn; O'Neil, Adam; Patel, Neal; Whitehead, Daniel C.; and Whitehead, Kristi J., "Exploiting the Gastrointestinal Microbiota as a Therapeutic Target for Type 1 Diabetes" (2015). *Focus on Creative Inquiry*. 120.  
https://tigerprints.clemson.edu/foci/120

This Poster is brought to you for free and open access by the Research and Innovation Month at TigerPrints. It has been accepted for inclusion in Focus on Creative Inquiry by an authorized administrator of TigerPrints. For more information, please contact kokeefe@clemson.edu.
Authors
Bryn Davis, Katrina Ross, Halee Bryant, Kelsey Tackeberry, Caitlyn Blake-Hedges, Adam O’Neil, Neal Patel, Daniel C. Whitehead, and Kristi J. Whitehead

This poster is available at TigerPrints: https://tigerprints.clemson.edu/foci/120
Exploiting the Gastrointestinal Microbiota as a Therapeutic Target for Type 1 Diabetes

Bryn Davis, Katrina Ross, Halee Byant, Kelsey Tackeberry, Caitlyn Blake-Hedges, Adam O’Neil, Neal Patel, Daniel C. Whitehead1,2, and Kristi J. Whitehead*1

1. Department of Biological Sciences, Clemson University, Clemson, SC; *whiteh1@clemson.edu
2. Department of Chemistry, Clemson University, Clemson SC

Introduction

Objective: To investigate a novel therapeutic for the treatment of Type 1 Diabetes (T1D).

The therapeutic would not kill, but just retard the growth of bacteria that are members of the genus Bacteroides while not effecting other bacterial species.

Why: T1D is a progressive autoimmune disorder characterized by the destruction of the insulin secreting Beta cells in the pancreas (Harrison et al. 2008).

While it has been previously acknowledged that there are genetic factors responsible for the onset of T1D, there is evidence to suggest environmental causes as well (Achenbach et al., 2005; Gale, 2002).

Not everyone that has the genetic predisposition to developing the disease will have T1D.

Our interest lies in the observation that just before the onset of T1D in an individual, there is a bloom of bacteria from the Bacteroides phyla. (Giongo et al., 2011).

How: We are investigating therapeutics that can inhibit the Starch Utilization System (SUS) specific to members of Bacteroides. Removing the SUS as a method of gaining biomass and energy for the cell would not kill the cell but slow its growth. The cell will have to find alternative and less effective methods of metabolism.

Current Work: We have investigated the effects of the drugs Acarbose and Migtitol on the growth of members from the Firmicutes and Bacteroides phyla.

Acarbose has been used previously in diabetes treatments because of its ability to retard the degradation of starch via inhibiting human α-amylases, α-glucosidases, similar to the ones observed in humans, can be found within the Sus.

Methods and Materials

• Bacteroides thetaiotaomicron was grown on tryptone yeast glucose (TYG) agar anaerobically.
• Colonies from plates were used to prepare overnight cultures of B. thetaiotaomicron TYG broth (grown anaerobically).
• Test tubes were filled with minimal media containing either 0.5% glucose, maltose, or pullulan as a carbon source, cysteine, Bacteroides culture, and varying amounts of acarbose.
• An anaerobic environment was created by inserting a cotton ball in the tube and lighting it on fire.
• Upon completion of burning, sodium bicarbonate and pyrogalol were added.
• The tube was stoppered, sealed, and incubated at 37°C for 24 hours.
• Inhibition was indicated the following day by measuring the optical density at a wavelength of 600nm.

• Lactobacillus reuteri strains were grown on de Man, Rogosa and Sharpe (MRS) agar aerobically.
• Colonies from plates were used to prepare overnight cultures of L. reuteri MRS broth (grown aerobically).
• Test tubes were filled with MRS broth containing either 0.5% glucose, maltose, or pullulan as a carbon source, cysteine, Lactobacillus culture, and varying amounts of acarbose.
• The test tubes were incubated at 37°C for 24 hours.
• Inhibition was indicated the following day by measuring the optical density at a wavelength of 600nm.

Preliminary Results

Bacteroides representative: B. ovatus, B. theta, B. fragilis
Firmicutes representatives: L. reuteri ATCC 55730 and L. reuteri PTA 6475

Monosaccharide representative: Glucose
Disaccharide representative: Maltose
Poly saccharide/Glycan representative: Pullulan

Table 1. Inhibition of Bacterial Growth by Acarbose

<table>
<thead>
<tr>
<th>Species</th>
<th>Glucose</th>
<th>Maltose</th>
<th>Pullulan</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. theta</td>
<td>None</td>
<td>At 200μM</td>
<td>At 50μM</td>
</tr>
<tr>
<td>B. fragilis</td>
<td>None</td>
<td>Not tested</td>
<td>At 10μM</td>
</tr>
<tr>
<td>L. reuteri ATCC 55730</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>L. reuteri PTA 6475</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

Conclusions

What we have found:
• Acarbose inhibits the growth of Bacteroides spp. and is not lethal to the cells.
• Acarbose does not inhibit Lactobacillus growth.
• Acarbose has structural similarities to many microbial molecules and is most likely effecting the Sus and therefore prohibiting its ability to breakdown available glycan molecules.

These findings have the possibility of providing a novel T1D treatment by preventing the bloom of Bacteroides in the large intestine.

Our future directions include:
• Assay inhibition on other complex carbohydrates
• Investigate inhibitory effects with another Bacteroides sp. and a non-Bacteroides sp. within the Bacteroides phylum
• Establish exact mode of inhibition of Sus system in B. thetaiotaomicron (i.e. where does it bind to inhibit?)
• Determine if correlation is an indicator of causation by conducting in vivo experiments using the Non-obese diabetic (NOD) mouse model to examine the effectiveness of acarbose administration at delaying or preventing the onset of T1D.

Acknowledgements

Eric Martens and Nicole Konopatik (University of Michigan) for donation of Bacteroides stocks
Clemson University Department of Biological Sciences
Creative Inquiry Program

References


Figure 1. Variation of ratios of the two major phyla of bacteria, Firmicutes and Bacteroides, found in 386 fecal samples

Figure 2. Molecular structure of D-glucose

Figure 3. Molecular structure of Migtitol

Figure 4. Molecular structure of Acarbose

Figure 5. Starch Utilization System (SUS) for B. thetaiotaomicron. This is the pathway responsible for colonization, degradation, and importation of complex carbohydrates

Figure 6. Representative growth assay demonstrating acarbose inhibition of B. theta and B. fragilis with varying levels of acarbose.

Figure 7. Anaerobic cultures of B. theta and B. fragilis with varying levels of acarbose.