Impact of ascending dosages of cadazolid or vancomycin on the intestinal microbiome during treatment of Clostridium difficile infection

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ABSTRACT

Background. To assess the microbiota sparing properties of cadazolid, fecal samples retained during the conduct of the phase 2 clinical study [NCT01222702] were cultured for Clostridium difficile count reductions and tested post hoc for quantification of major microbiota groups with qPCR and 16s sequence analysis using next generation sequencing (NGS).

Methods. Eligible subjects with primary or first recurrences of C. difficile infection (CDI) were randomized to receive 250 mg bid, 500 mg bid, or 1000 mg bid of cadazolid, or to receive vancomycin 125 mg qid, each for 10 days. Paired day 0 and day 13 fecal samples from 40 patients, 10 subjects in each treatment group, were serially diluted 10⁻²,4,6 and plated onto CCFA agar. qPCR was performed using primer pairs for C. difficile and 8 different major intestinal bacterial groups. For NGS the V3 region of the 16s rDNA gene was PCR amplified and sequenced on the Illumina MiSeq platform.

Results. All dosages of cadazolid and vancomycin significantly reduced C. difficile counts, as measured by quantitative culture (p<0.01) and qPCR (p<0.01). qPCR analysis showed that escalating dosages of cadazolid did not reduce Bacteroides, Clostridium clusters XIVa and IV counts, whereas cadazolid 500 mg and 1000 mg bid reduced Bifidobacterium counts (p<0.02). Vancomycin treatment significantly reduced all of the foregoing groups (p<0.02). Using NGS, cadazolid treatment groups had lower numbers of significantly different operational taxonomic units (OTUs) between day 0 and 13 compared to vancomycin. Cadazolid day 13 samples had no change in α-diversity while vancomycin day 13 samples had significantly lower Shannon Diversity Index relative to the day 0 samples. Vancomycin samples were characterized by large drops in the Lachnospiraceae, Clostridiaceae, Bacteroidaceae and Prevotellaceae families and increases in Enterobacteriaceae, Veillonellaceae, and Lachnocloaceae.

Conclusion. These findings indicate that CDI subjects treated with cadazolid have less alteration to the fecal microbiome than CDI subjects treated with vancomycin. Combined with clinical outcomes, the microbial ecologic data support the selection of cadazolid 250 mg bid dosage being investigated in phase 3 clinical trials.

INTRODUCTION

Treatment of the majority of patients who develop C. difficile infection remains suboptimal because of the non-selectivity and limited potency of standard treatments using metronidazole and vancomycin (McFarland, 2005. J Med Microbiol 54: 101-111; Johnson et al., 2014. Clin Infect Dis 59: 345-354; Shields et al., 2015. Anaerobe 34: 59-73). Both metronidazole and vancomycin are damaging to the normal microbiota, contributing to ~20-24% risk of recurrence due to the disturbed microbiome (Lowe et al., 2015. Clin Infect Dis 60 Suppl 2: S51-7). Therefore, new treatments for CDI must both kill the pathogens and at the same time spare destruction of the protective host microbiota. In this study we build on published clinical data looking at the safety and efficacy of cadazolid for treating CDI patients, in phase 2 clinical trials cadazolid had higher sustained clinical response rates (46.7-60.0%) compared with vancomycin (33.3%) (Lowe et al. 2015. Antimicrob Agents Chemother 59:4266-73).

AIM

To assess the microbiota sparing properties of cadazolid, fecal samples retained at the Calgary site during the conduct of the phase 2 clinical study [NCT01222702] were cultured for C. difficile and tested post hoc for quantification of major microbiota groups.

METHODS AND PATIENTS

Quantitative C. difficile counts, total, and sparse, were performed on Cycloserine Cefoxitin Fructose Agar (CCFA). DNA was extracted from frozen stool using the Zymo ZR Fecal DNA Stool MiniPrep Kit and used for both quantification of targeted bacterial DNA by qPCR and 16s rDNA profiling using next generation sequencing (NGS). For culture and qPCR data were used to calculate significant differences between groups.

Biostatistical analysis was performed in R using functions from phyloseq, DESeq2 and vegan. Eligible subjects with primary or first recurrences of CDI were randomized to receive 250, 500 or 1000 mg bid of cadazolid, or to receive vancomycin 125 mg qid, each for 10 days. Stool samples were collected at day 0, 3, 5 and 13.

RESULTS

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α-diversity in the microbiome of CDI patients. The Principle coordinate analysis (PCoA) is based on Bray-Curtis distance metric and non-metric multidimensional scaling (NMDS) and allows visualization of differences between groups. This ordination had a stress value of 0.178.

Figure 3. The microbiome of vancomycin- but not cadazolid-treated patients separates into two distinct groups before and after antibiotic treatment. The Principle coordinate analysis (PCoA) is based on Bray-Curtis distance metric and non-metric multidimensional scaling (NMDS) and allows visualization of differences between groups. The microbiome of vancomycin- but not cadazolid-treated patients separates into two distinct groups before and after antibiotic treatment. The Principle coordinate analysis (PCoA) is based on Bray-Curtis distance metric and non-metric multidimensional scaling (NMDS) and allows visualization of differences between groups.

Figure 4. Cadazolid treatment does not significantly alter α diversity in the microbiome of CDI patients. α diversity of 16s rDNA microbiome was measured using the Shannon index. Statistical comparisons were performed using a two-sided Mann-Whitney test. A false discovery rate correction was applied to correct for multiple testing.

Figure 5. Vancomycin alters abundance of more OTUs and bacterial families relative to all doses of cadazolid. Classification of differentially abundant OTUs by genus. Mean log, fold-change for families that showed a significant difference (p<0.01) between Day 0 and Day 13. Differential abundance analysis was performed using a generalized linear model in DESeq2 (Love et al. 2014. Genome Biol 15(12):550).

Table 1. Differentially abundant OTUs for each drug treatment at day 13 relative to day 0.

<table>
<thead>
<tr>
<th>Drug and dose</th>
<th>Number of OTUs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cadazolid 250 bid</td>
<td>27</td>
</tr>
<tr>
<td>Cadazolid 500 bid</td>
<td>52</td>
</tr>
<tr>
<td>Cadazolid 1000 bid</td>
<td>62</td>
</tr>
<tr>
<td>Vancomycin 125 bid</td>
<td>80</td>
</tr>
</tbody>
</table>

CONCLUSION

In phase 2 clinical trials higher sustained clinical response rates were observed with cadazolid compared to vancomycin. The data presented here identifies the microbiome-sparing properties of cadazolid and increases our understanding of the microbiophage behind the observed clinical response rates and sustained cure between cadazolid and vancomycin.

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