Rifaximin Is Associated With Modest, Transient Decreases in Multiple Taxa in the Gut Microbiota of Patients With Diarrhea-Predominant Irritable Bowel Syndrome

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BACKGROUND

- Irritable bowel syndrome (IBS) is a functional bowel disorder characterized by recurrent abdominal pain associated with defecation or altered bowel habits[1]
- The pathophysiology of IBS remains to be elucidated; however, the gut microbiota may play a role, given the association between enteric infection and development of postinfectious IBS,[2] quantitative and qualitative differences in the gut microbiota of patients with IBS versus healthy individuals,[3] and improvement of symptoms of IBS observed in some patients following therapy with antibiotics[4]
- Rifaximin is an oral, nonsystemic, gastrointestinal (GI)-targeted antibiotic indicated in the United States for the treatment of IBS with diarrhea (diarrhea-predominant IBS [IBS-D]) in adults[5]
- Rifaximin appears to have several effects beyond its *in vitro* activity against gram-negative and gram-positive bacteria,[6] including effects on mucosal inflammation and visceral hypersensitivity, bacterial colonization of the GI tract, and bacterial virulence factors[7-9]
  - The efficacy and safety of rifaximin have been demonstrated in several clinical trials[10,11]
- Given the emerging association between IBS and the gut microbiota, this study evaluated the impact of repeat treatment with rifaximin on the gut microbiota in patients with IBS-D

AIM

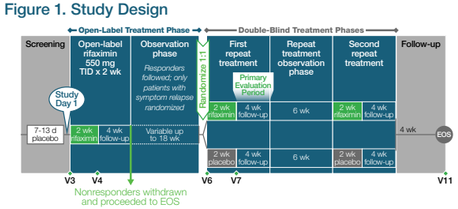
- To examine potential effects of rifaximin on the GI microbial community in patients with IBS-D

METHODS

Study Design and Patients

- Adults with IBS-D (Rome III criteria) were treated with rifaximin 550 mg three times daily (TID) for 2 weeks followed by a 4-week treatment free period to assess response; responders were followed for up to 18 additional weeks; those who experienced relapse were randomly assigned to receive two 14-day double-blind repeat treatments with rifaximin 550 mg TID or placebo TID (**Figure 1**); double-blind repeat treatment courses were separated by 10 weeks
  - Exclusion criteria included taking probiotics, rifaximin, or any other antibiotic within 14 days of providing written informed consent
  - Response was defined as simultaneously meeting weekly response criteria for abdominal pain (≥30% improvement from baseline in mean weekly abdominal pain score) and stool consistency (≥50% decrease from baseline in number of days/week with Bristol Stool Scale type 6 or 7 stools) for ≥2 of the first 4 weeks after treatment

Figure 1. Study Design



EOS = end of study; TID = three times daily; V = clinic visit.
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METHODS

- All patients consented to providing stool samples, and patients (responders and nonresponders) were randomly selected for inclusion in the current substudy
- Fresh stool samples were collected prospectively from patients at clinic visits (V) before (open-label baseline; V3) and after open-label treatment with rifaximin (open-label week 2; V4), before (double-blind baseline; V6) and after the first double-blind treatment with rifaximin or placebo (double-blind week 2; V7), and at the end of the study (V11; **Figure 1**)
  - Patients who were unable to provide fresh samples at clinic visits were offered a stool collection kit for home collection; they were instructed to refrigerate and return their samples to the clinic as soon as possible

Genomic Testing

- Stool samples were frozen and shipped to Covance Genomics (Seattle, WA) for genomic sequencing
- Samples were thawed at time of testing; DNA was extracted from the samples using the QIAamp® DNA Stool Mini Kit (Qiagen, Germantown, MD)
- The variable 4 hypervariable region of the 16S ribosomal RNA (rRNA) gene was sequenced with the HiSeq 2500 System (Illumina, Inc., San Diego, CA) using a polymerase chain reaction amplicon of 286 base pairs generated using forward primer F515 (5'-GTGCCAGCMGCCGCGGTAA-3') and reverse primer R806 (5'-GGACTACHVGGGTWTCTAAT-3')

Data Analysis

- The Ribosomal Database Project (RDP) algorithm was applied to each paired read with a threshold of 50%,[12] with RDP calls used at the family level for reliability
  - Sequences were included for analysis when the RDP algorithm produced an identical call on both nonoverlapping paired end reads
- Data included in the diversity index reflected measures of richness (number of families in each sample corrected for different samples having different numbers of sequences), evenness (calculated by Shannon's equitability, with values ranging from 0 to 1 [complete evenness]), and Shannon diversity (measure of overall community complexity)

Statistical Analysis

- RDP counts to family level were log-normalized per methods described in Sanapareddy et al[13]
  - Taxa relative to abundances represented the $\log_{10}$ sequenced count for each sample, with normalization to ensure the total number of sequence counts was identical for all samples
- Statistical models were built for taxa present in ≥10% of samples
- Correction for multiple hypothesis testing was performed with the Benjamini–Hochberg procedure using the p.adjust function in R; multidimensional scaling ordination was performed using the capscale function in the Vegan package in R (Oksanen et al, The R Foundation, Vienna, Austria) with Bray–Curtis distance
- Supervised classification was performed using the support vector machine (SVM)^{light} package (Joachims T, Cornell University, Ithaca, NY) with the default parameters

RESULTS

Patients and Isolated Sequences

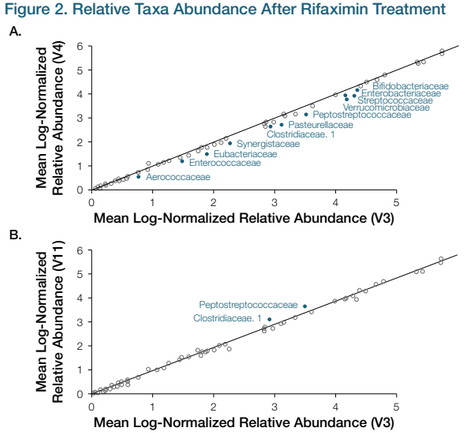
- Of the 103 patients randomly selected for inclusion in the stool microbiota analysis, 37 and 36 patients received double-blind rifaximin and placebo, respectively; 30 patients received only open-label rifaximin
  - Most of the 103 patients were white (82.5%) and female (73.8%), with a median age of 48.0 years (range, 19-85 years); demographic characteristics were comparable between the double-blind groups
- A total of 675 stool samples were included in the analysis, generating 2,309,172,633 paired-end 16S rRNA sequence reads after high-throughput sequencing
  - A total of 1,868,592,999 reads (~81% of all sequenced paired ends) were called by the RDP algorithm to family at the 50% confidence level with an identical call on both nonoverlapping paired ends and included for downstream analysis
  - 666 stool samples representing 440 combinations of patients and time points were included in the analysis (**Table 1**)

Table 1. Number of Samples Sequenced From Stool Samples Submitted at Each Visit

Visit	Samples, n*
Open-label baseline (V3)	101
Open-label week 2 (V4)	102†
Double-blind baseline (V6)	69
Double-blind week 2 (V7)	72
End of study (V11)	96

*A total of 103 patients with diarrhea-predominant irritable bowel syndrome were included in the study, but no patients had samples evaluated at all time points.
†Individual patients could have had >1 sample evaluated at a given time point.
V = clinic visit.

Figure 2. Relative Taxa Abundance After Rifaximin Treatment



Mean relative abundance of 74 non-rare taxa observed in ≥10% of all samples at (A) open-label baseline (V3) and after 2 weeks of open-label rifaximin (V4); and (B) at open-label baseline (V3) and at the end of the study (V11). The closed blue circles indicate taxa that were significantly different in paired Wilcoxon comparisons. The black lines are the identity lines.
V = clinic visit.

Effects of Open-Label Rifaximin on Gut Microbiota

- Overall, the gut microbial community was stable following 2-week treatment with open-label rifaximin
  - Eleven taxa had significantly lower relative abundance at a 10% false discovery rate threshold (**Figure 2A**)
  - Rifaximin effects were generally short-term, as there were few significantly different changes in taxa relative abundance at the end of the study (up to 46 weeks after open-label baseline) compared with what was observed at open-label baseline (**Figure 2B**)

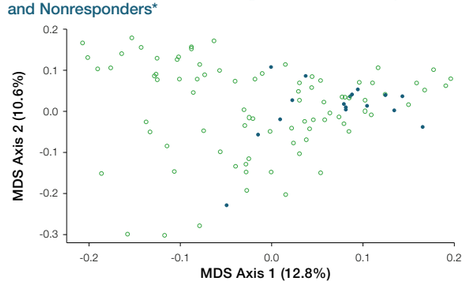
Effects of Double-Blind Rifaximin on Gut Microbiota

- Overall, there were no substantial differences in the gut microbial community following double-blind repeat treatment with rifaximin or placebo
  - However, at a simple uncorrected value of $P<0.05$, 9 taxa differed significantly between V6 (double-blind baseline) and V7 (double-blind week 2) for rifaximin, including *Peptostreptococcaceae* and *Clostridiaceae*; only 1 taxon differed significantly for placebo during this time frame

Effects of Rifaximin on Gut Microbiota as a Predictor of Response

- Of the 101 patients with IBS-D included in the analysis, 84 and 17 patients were responders and nonresponders, respectively, to open-label rifaximin treatment
- At V3 (open-label baseline), 9 taxa differed significantly at a 10% false discovery rate (unpaired Wilcoxon test) between responders and nonresponders
- The state of the GI microbial community for each patient at baseline had modest but statistically significant power to predict response to rifaximin treatment ($P=0.001$; **Figure 3**)
  - Using the supervised classifier SVM^{light} and dividing the dataset into 2 roughly equal-sized groups (7-10 responders in a training set), the average area under the receiver operating characteristic (ROC) curve was 0.62; across 250 permutations this was significantly different ($P=2.8 \times 10^{-20}$, paired Wilcoxon test) from areas under ROC curves generated by randomly permuting the responder and nonresponder assignments in the training set

Figure 3. Multidimensional Scaling Ordination for Responders and Nonresponders*



*Data for open-label baseline (clinic visit 3) samples.
Open green circles represent responders; closed blue circles represent nonresponders.

CONCLUSIONS

- **The results of this study, the first to measure response of the gut microbial community following repeat rifaximin treatment, suggest that rifaximin has a modest, largely transient effect on a range of stool microbes**
- **Future studies may determine whether the taxa affected by rifaximin are causally associated with IBS-D**

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