Kidney360

Randomized, Placebo-Controlled Trial of Rifaximin Therapy for Lowering Gut-Derived Cardiovascular Toxins and Inflammation in CKD

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Abstract

Background Recent evidence suggests the systemic accumulation of by-products of gut microbes contributes to cardiovascular morbidity in patients with CKD. Limiting the generation of toxic bacterial by-products by manipulating the intestinal microbiota may be a novel strategy for reducing cardiovascular disease in CKD. Rifaximin is a minimally absorbed, oral antibiotic that targets intestinal pathogens and is commonly used as chronic therapy for the prevention of encephalopathy in patients with cirrhosis.

Methods We conducted a randomized, double-blinded, placebo-controlled trial to determine the effect of a 10-day course of oral rifaximin 550 mg BID versus placebo on circulating concentrations of gut-derived cardiovascular toxins and proinflammatory cytokines in patients with stage 3–5 CKD (n=38). The primary clinical outcome was change in serum trimethylamine *N*-oxide (TMAO) concentrations from baseline to study end. Secondary outcomes included change in serum concentrations of p-cresol sulfate, indoxyl sulfate, kynurenic acid, deoxycholic acid, and inflammatory cytokines (C-reactive protein, IL-6, IL-1β), and change in composition and diversity of fecal microbiota.

Results A total of 19 patients were randomized to each of the rifaximin and placebo arms, with n=17 and n=14 completing both study visits in these respective groups. We observed no difference in serum TMAO change (post-therapy minus baseline TMAO) between the rifaximin and placebo groups (mean TMAO change −3.9±15.4 for rifaximin versus 0.5±9.5 for placebo, P=0.49). Similarly, we found no significant change in serum concentrations for p-cresol sulfate, indoxyl sulfate, kynurenic acid, deoxycholic acid, and inflammatory cytokines. We did observe differences in colonic bacterial communities, with the rifaximin group exhibiting significant decreases in bacterial richness (Chao1, P=0.02) and diversity (Shannon H, P=0.05), along with altered abundance of several bacterial genera.

Conclusions Short-term rifaximin treatment failed to reduce gut-derived cardiovascular toxins and inflammatory cytokines in patients with CKD.

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Introduction

Patients with CKD demonstrate a disproportionate burden of cardiovascular disease compared with individuals with normal kidney function (1–5), which largely drives the excess morbidity and mortality observed in this group. Traditional risk factors alone fail to explain the extensive cardiovascular pathology that occurs in patients with CKD, implying that unique cardiovascular risk factors emerge with a loss of kidney function (6,7).

Bacterial endotoxin (*i.e.*, LPS), which is suspected to be of intestinal origin, is present in the bloodstream of patients with advanced CKD (8). It is postulated that this endotoxemia stimulates innate immune responses

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and proinflammatory pathways that contribute to excess cardiovascular pathology in this population. Moreover, a variety of gut-derived bacterial metabolites with purported cardiovascular toxicity accumulate in the circulation of patients with CKD (9–14). P-cresol sulfate, indoxyl sulfate, and trimethylamine *N*-oxide (TMAO) are well-established examples of such metabolites (9). In addition, deoxycholic acid is a secondary bile acid generated by gut bacteria that is elevated in CKD and has been recently associated with vascular calcification (12,15). Likewise, kynurenic acid is a tryptophan metabolite that is generated under substantial influence by gut microbes (16), is associated with adverse cardiovascular outcomes in non-CKD populations (17), and exhibits serum concentrations that are increased in CKD (10).

Despite significant data to suggest a potential contribution of these compounds to cardiovascular disease, effective therapies for lowering the production of these potentially toxic metabolites are lacking (9,14). Therapies that focus on decreasing the generation of these bacterial metabolites and suppressing chronic inflammation could be a novel strategy for limiting cardiovascular pathology in these patients. Prior studies have suggested that suppressing the gut microbiota with antibiotics can dramatically lower the generation of several bacterial metabolites (18–20); however, administration of broad-spectrum antibiotics with systemic activity is not feasible as chronic therapy.

Rifaximin is a minimally absorbed, oral antibiotic that is concentrated in the gastrointestinal tract and demonstrates bactericidal and bacteriostatic activity against both Gram-positive and Gram-negative aerobic and anaerobic bacteria (21). In patients with advanced liver disease, rifaximin has shown efficacy as chronic therapy to suppress recurrent hepatic encephalopathy that partially stems from the systemic accumulation of neurotoxins derived from intestinal bacteria (22). Rifaximin is unique in that chronic administration appears to result in very limited disruption of the overall balance of the intestinal microbiome, while possibly enhancing the presence of symbiotic bacteria that promote intestinal health (23–26). Thus, in this study, we conducted a prospective, randomized, placebo-controlled trial to investigate the effect of short-term rifaximin therapy on circulating concentrations of bacterial metabolites and inflammatory cytokines with purported cardiovascular toxicity, along with the relative abundance of fecal microbial communities in patients with CKD.

Materials and Methods

Study Participants

Participants were recruited from the University of Kansas Medical Center (KUMC) outpatient nephrology clinics from June 2015 to January 2017; we ended recruitment after meeting the prespecified enrollment target. Inclusion criteria included being ≥18 years of age and having CKD with eGFR of ≤39 ml/min per 1.73 m^2. Exclusion criteria included the inability or unwillingness to provide consent, patients receiving chronic dialysis, prior organ transplantation, pregnancy, hemodynamic instability, liver disease, pancreatic insufficiency, inflammatory bowel disease, active infection within the last month, history of *Clostridium difficile* infection, known abnormal bowel anatomy, current use of specific medications (immunosuppressants, bile acid sequestrants, antidiarrheal agents), and use of antibiotics within 3 months.

Study Design

This was a prospective, randomized, double-blinded, parallel clinical trial of 550 mg rifaximin versus placebo by mouth, twice a day, for 10 days in 38 patients (19 per arm) with advanced CKD (eGFR ≤39 ml/min per 1.73 m^2). The 10-day duration of therapy was chosen on the basis of prior studies showing: (*1*) a 7-day course of broad-spectrum antibiotics dramatically decreased circulating TMAO in healthy humans (18); (*2*) a 7-day course of rifaximin lowered serum TMAO in CKD rodents (unpublished observation); and (*3*) a 7- to 10-day course of rifaximin in humans with irritable bowel syndrome from small intestine bacterial overgrowth has been associated with a reduction in irritable bowel syndrome symptoms and improvement in small intestine bacterial overgrowth measurements (27,28). The 550 mg twice per day dosage was chosen on the basis of data from a prominent clinical trial demonstrating efficacy of this regimen for the prevention of hepatic encephalopathy in patients with cirrhosis (22). The eGFR cutoff of ≤39 ml/min per 1.73 m^2 was derived from published data showing a dramatic increase in serum TMAO at an eGFR below this value (11).

The KUMC Investigational Pharmacy randomized patients in a 1:1 ratio using a computer-generated, random assignment program and dispensed the study drug. The physician investigators and study coordinators who handled enrollment procedures, sample collection/processing, and study documentation were blinded to treatment assignment for the entirety of the trial. Study drugs were compounded into identical-appearing formulations by a local compounding pharmacy. Pill bottles were collected at the final study visit, and medication compliance was calculated as the percentage of missing tablets divided by the total tablets provided. Fasting blood and stool samples were collected at both study visits, processed immediately, and stored at −80°C until measurements were conducted. Fecal samples were collected by study participants at home within 24 hours of their study visits. Specimen containers were put in a sealed biohazard bag, immediately placed in the participant's home freezer, and then transported on ice to the study visit to preserve sample quality. Figure 1 outlines the study design implemented for this investigation.

The primary end point was change in serum TMAO concentrations from baseline to study end (day 11). Secondary outcomes included change in serum concentrations of p-cresol sulfate, indoxyl sulfate, kynurenic acid, deoxycholic acid, C-reactive protein (CRP), IL-6, and IL-1β, along with change in fecal bacterial composition. The study was conducted according to the guidelines outlined in the Declaration of Helsinki, and all procedures involving human participants were approved by the Human Subjects Committee at KUMC. Written informed consent was obtained from all participants.

Metabolite Quantification

Serum TMAO concentrations were measured by ultra-high-performance liquid chromatography–tandem mass

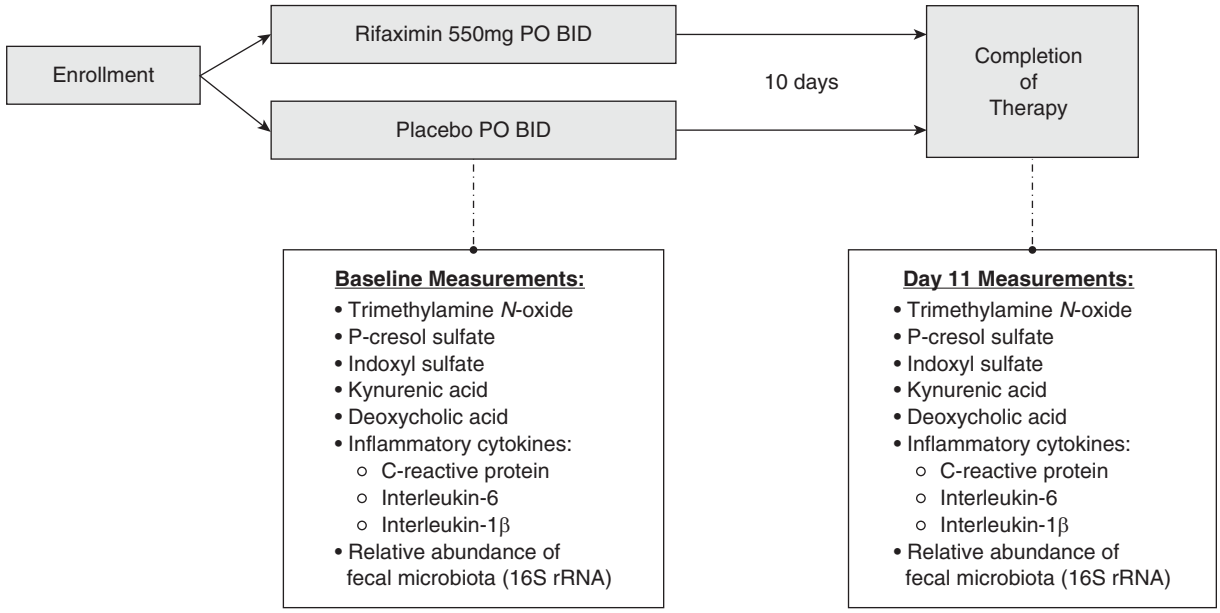

Figure 1. | **Graphical representation of study design showing treatment schedule, timing of biological sample collection, and study endpoints.** BID, twice a day; PO, by mouth; rRNA, ribosomal RNA.

spectrometry (LC-MS/MS) using heated electrospray ionization (positive mode) and selected reaction monitoring, as previously described (29). The standard curve ranged from 0.010 to 5.00 μg/ml (0.13–66.6 μM). Samples that were above the upper limit of the standard curve were diluted in PBS. The within-run and between-run coefficient of variation was <10%.

Serum deoxycholic acid concentrations were quantified using LC-MS/MS as described in a prior report (30). In brief, 100 μl serum was diluted in 300 μl cold acetonitrile containing 3 ng of hexadeuterated-DCA (Cambridge Isotope Laboratory) as internal standard. The mixture was passed through a Phree Phospholipid Removal Plate (Phenomenex). The eluate was evaporated with nitrogen gas, and then redissolved in 100 μl of 10 mM ammonium acetate buffer (pH 8.0)/methanol (1:1, vol/vol). A 10-μl aliquot of each sample was then injected into the electrospray ionization LC-MS/MS system (QTRAP 3200; SCIEX).

Serum kynurenic acid, p-cresol sulfate, and indoxyl sulfate concentrations were quantified by LC-MS/MS, using previously published methods (13). Samples were precipitated in organic solvent, followed by solid-phase extraction (Phree Phospholipid Removal Plate). Dried extracts were reconstituted in 80 μl of 5% acetonitrile/0.2% formic acid in water and filtered through a large-pore filter plate (MSBVN1210; Millipore), to remove particulates, before introduction into a triple-quadrupole tandem mass spectrometer (6500; Sciex). Data were normalized to internal standard peak areas of labeled solutes. Calibration was achieved using a single-point calibration approach to account for drift, with five calibrator replicates included on each plate.

Measurement of serum inflammatory cytokines was performed using the following commercially available assays according to the manufacturer instructions: human CRP ELISA (catalog number ENZ-KIT102-0001; Enzo Life Sciences, Farmingdale, NY), high-sensitivity human IL-6 ELISA (catalog number BMS213HS; Thermo Fisher Scientific, Waltham, MA), and high-sensitivity IL-1β ELISA (catalog number BMS224HS; Thermo Fisher Scientific).

High-Throughput DNA Sequencing for Microbiome Analysis

Bacterial profiles were determined by broad-range amplification and sequence analysis of 16S ribosomal RNA (rRNA) genes, following our previously described methods (31–33). In brief, amplicons were generated using bar-coded primers targeting the V3V4 variable region of the 16S rRNA gene. Pooled amplicons were paired-end sequenced (2×300 nt) on the Illumina Miseq platform and processed, as previously described (31–33). Assembled sequences were aligned and classified with SINA (1.3.0-r23838) using the Silva 115NR99 database (34,35). Operational taxonomic units were produced by clustering sequences with identical taxonomic assignments. The median Good coverage score was ≥99.5% at the rarefaction point of 30,000 sequences.

Statistical Analyses

We calculated that 30 total subjects (15 per treatment arm) would allow for estimation of the difference between treatment groups in baseline to post-treatment change scores for TMAO, within approximately 0.75 SDs, using the formula for 95% confidence intervals for the two-sample *t* test. For the analysis of demographic data, the Pearson chi-squared statistic was used to measure the association between categoric variables among treatment groups. Continuous measurements between groups were assessed by unpaired, two-sample *t* test. Assumptions for normality were examined and, if not satisfied, a nonparametric Wilcoxon signed rank test was used to measure the continuous outcomes between groups. For the primary study end-point analysis, the estimated differences between the baseline and post-treatment

time points were compared by paired, two-sample *t* test and corresponding 95% confidence intervals generated by the *t* test; if assumptions were violated, an exact Wilcoxon rank sum test was implemented with 95% confidence interval generated by the Hodges–Lehmann estimation method. As a secondary analysis, an analysis of covariance model, with change scores as the outcome and baseline measurements as the explanatory factor, was conducted. Residuals were assessed in all models to evaluate model fit to underlying assumptions. $P<0.05$ was considered statistically significant for the outcomes of interest.

For stool analyses, only participants that provided samples at both visits were included ($n=12$ per group). Microbiome analyses used Explicet (version 2.10.5) and the R statistical software package (36). The relative abundance of each taxon was calculated as the number of 16S rRNA sequences of a given taxon divided by the total number of 16S rRNA sequences in a patient's sample. Differences in microbiome composition (*i.e.*, β-diversity) between subsets were assessed by a nonparametric, permutational multivariate ANOVA test using Bray–Curtis (weighted) and Jaccard (unweighted) dissimilarities; *P*-values were estimated through 1,000,000 permutations. The α-diversity indices were calculated in Explicet at a rarefaction point of 30,000 sequences through 1000 permutations. α-Diversity indices and relative abundances of individual operational taxonomic units were compared across groups through nonparametric longitudinal analysis, using the nparLD R package (37).

Results

Participant Demographic Characteristics and Study Enrollment

Table 1 lists the baseline demographic characteristics for each treatment group. We observed relatively equal between-group distributions for age, race, and body mass index; however, the placebo group did demonstrate a higher percentage of female participants and a greater prevalence of both diabetes and diabetic kidney disease. A comparison of between-group differences in baseline laboratory parameters revealed a higher baseline eGFR in the placebo group, but comparable severity of proteinuria. Baseline values for mean TMAO, p-cresol sulfate, deoxycholic acid, kynurenic acid, CRP, and IL-6 were relatively equal between groups; however, mean baseline indoxyl sulfate levels were higher for the rifaximin group. Of note, IL-1β was undetectable in the serum of most study participants, so data were not reported for this cytokine.

Figure 2 outlines the participant enrollment for this study. Of the 80 potential study participants screened, 38 provided written consent for study inclusion and were randomized. A

Table 1. Baseline demographics and laboratory values

Demographics and Laboratory Values	Rifaximin ($n=17$)	Placebo ($n=14$)	*P* Value
Demographics			
Age (yr)	62±13	65±9	0.52
Sex			0.22
Female	6 (35%)	8 (57%)	
Male	11 (65%)	6 (43%)	
Race			0.99
Black	3 (18%)	3 (21%)	
White	14 (82%)	9 (64%)	
Other	0 (0%)	2 (14%)	
Body mass index, kg/m^2	31±8	35±9	0.16
Diabetes (type 1/2)	7 (41%)	11 (79%)	0.07
Etiology of CKD			0.01
Diabetes mellitus	4 (24%)	10 (71%)	
GN	1 (6%)	0 (0%)	
Hypertension	4 (24%)	1 (7%)	
Polycystic kidney disease	0 (0%)	1 (7%)	
Other	8 (47%)	2 (14%)	
Baseline laboratory parameters			
eGFR (ml/min per 1.73 m^2)	27.9±10.6	34.4±14.2	0.16
Trimethylamine *N*-oxide (μM)	18.8±18.7	15.6±11.6	0.74
P-cresol sulfate (μg/ml)	18.5±10.0	14.4±4.7	0.49
Indoxyl sulfate (μg/ml)	3.7±2.0	2.3±0.8	0.02
Kynurenic acid (ng/ml)	28.1±21.3	19.6±19.0	0.20
Deoxycholic acid (ng/ml)	372.2±268.3	609.8±384.7	0.14
C-reactive protein (μg/ml)	8.5±22.1	8.2±11.6	0.32
IL-6 (pg/ml)	2.1±1.6	2.6±1.8	0.08
Proteinuria			0.72
None[a]	6 (35%)	6 (43%)	
Mild[b]	4 (24%)	4 (29%)	
Moderate[c]	7 (41%)	4 (29%)	

Continuous variables presented as mean±SD; categoric variables presented as *n* (%).
[a]Urine protein-creatinine ratio ≤0.3 or negative urinalysis.
[b]Urine protein-creatinine between 0.3 and 1.0, or urine dipstick of trace to 1+.
[c]Urine protein-creatinine ratio ≥1.0 or urine dipstick >1+.

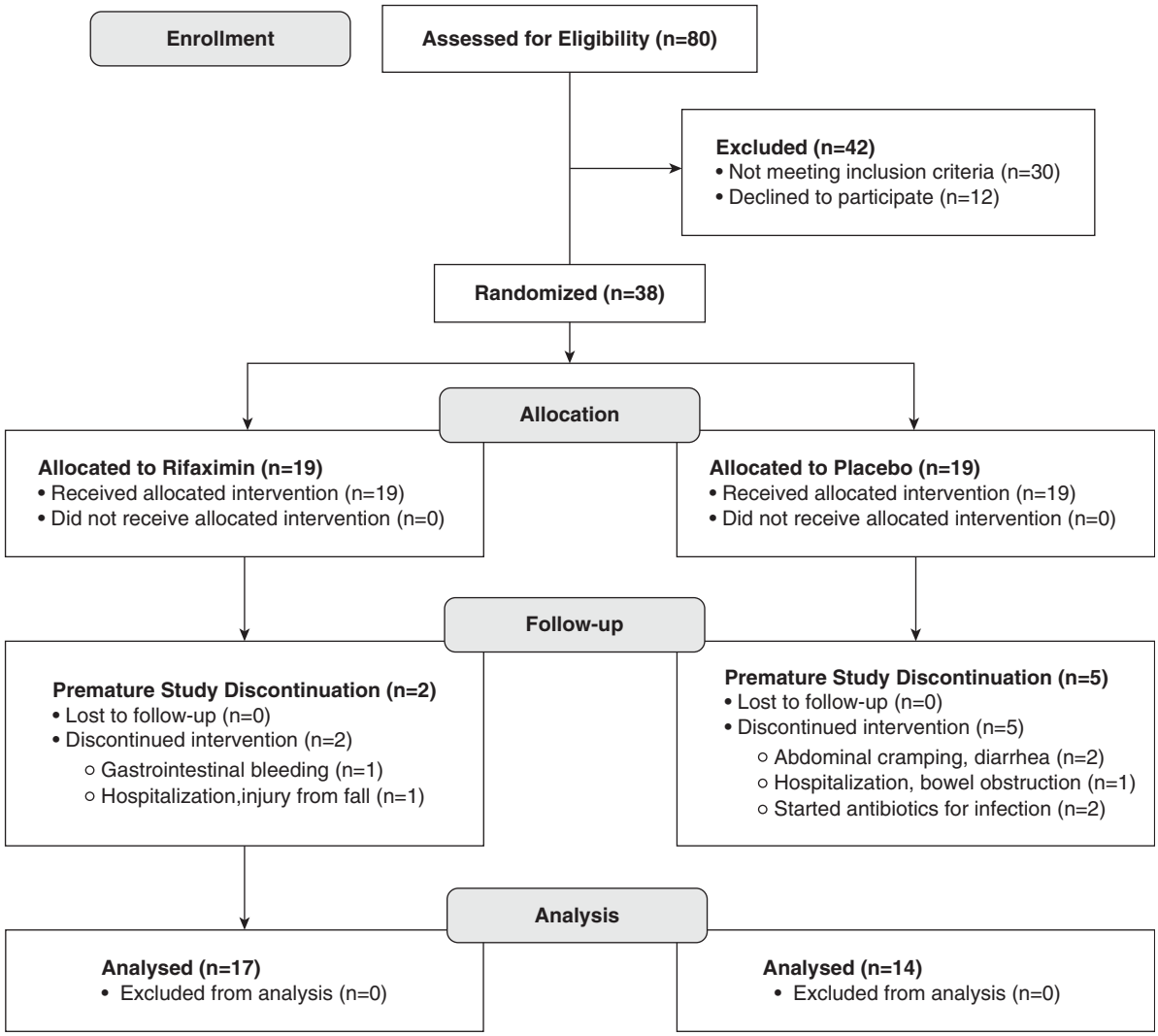

Figure 2. | **Graphical representation of study participant enrollment, randomization and disposition.**

total of 17 participants completed rifaximin therapy, and 14 participants completed placebo therapy. Reasons for participant dropout included gastrointestinal symptoms, start of alternative antibiotic therapy, hospitalization for fall, and hospitalization for mechanical bowel obstruction (unrelated to therapy). Study drug compliance, determined on the basis of final-visit pill counts, was 99% for the rifaximin group and 96% for the placebo group.

Table 2. Serum metabolite and inflammatory cytokine concentrations

Parameter	Rifaximin Group			Placebo Group		
	Pretreatment	Post-treatment	Change	Pretreatment	Post-treatment	Change
Trimethylamine *N*-oxide (μM)	18.8±18.7	14.9±10.2	−3.9±15.4	15.6±11.6	16.1±13.4	0.5±9.5
p-Cresol sulfate (μg/ml)	18.5±10.0	14.2±8.8	−4.3±7.6	14.4±4.7	15.3±8.2	0.9±7.6
Indoxyl sulfate (μg/ml)	3.7±2.0	3.8±2.6	0.1±1.4	2.3±0.8	2.3±0.9	0.0±1.0
Kynurenic acid (ng/ml)	28.1±21.3	28.5±15.6	0.4±10.6	19.6±6.9	19.0±6.1	−0.6±2.6
Deoxycholic acid (ng/ml)	372.2±268.3	332.5±294.6	−39.7±275.1	609.8±384.7	433.1±226.5	−176.7±291.3
C-reactive protein (μg/ml)	8.5±22.1	14.5±37.4	6.0±19.7	8.2±11.6	5.7±6.0	−2.6±5.8
IL-6 (pg/ml)	2.1±1.6	2.4±1.8	0.3±1.1	2.6±1.8	3.4±2.6	0.8±2.1

All measurements presented as mean±SD.

Change in Serum Concentrations of Bacterial Metabolites and Inflammatory Cytokines

Table 2 lists the mean and SD for pre- and post-therapy serum concentrations of gut-derived metabolites and inflammatory cytokines. Figure 3 depicts both the individual values and the absolute change in these measurements for each study participant. We observed no statistically significant change in the serum concentration for all measured metabolites and cytokines after rifaximin or placebo therapies.

Change in Stool Bacterial Composition

Microbiome profiling using 16S rRNA gene sequencing was conducted on stool samples from baseline and post-treatment. We found rifaximin treatment reduced the richness (Chao1, $P<0.02$) and diversity (Shannon H, $P=0.05$) of bacterial taxa compared with placebo (Figure 4A), whereas evenness (Shannon H/Hmax; Hmax = maximum Shannon H for a sequence library) was not significantly altered ($P=0.52$). In contrast, overall community composition (β-diversity) did not differ between treatment groups ($P>0.05$ for all comparisons of groups by time; Figure 4B). Nevertheless, ten bacterial taxa, all of which were members of the Gram-positive phyla Firmicutes or Actinobacteria, were significantly reduced (nominal $P<0.05$) by rifaximin treatment compared with placebo (Table 3). Three of these genera, *Clostridium*, *Turicibacter*, and *Anaerotruncus*, remained significant after false-discovery-rate correction (false discovery rate<0.1).

Discussion

CKD is an independent risk factor for cardiovascular morbidity and mortality, which may be partially explained by the presence of nontraditional risk factors for cardiovascular pathology in patients with CKD. Decrements in kidney function are accompanied by intestinal dysbiosis and defects in intestinal barrier function, which may contribute to the accumulation of bacterial endotoxins and metabolites in the bloodstream that may trigger innate immune responses or directly promote cardiovascular injury (9). As a result, there is considerable interest in exploring how manipulations of the gut microbiome in patients with CKD may affect cardiovascular risk.

The current investigation tested the effect of rifaximin on circulating concentrations of potential cardiovascular toxins derived from gut microbes. Rifaximin is an oral antibiotic that targets intestinal bacteria and is currently approved for the prevention of hepatic encephalopathy, which partially results from the accumulation of gut-derived neurotoxins in patients with cirrhosis (22). Rifaximin is a unique antibiotic in that chronic administration results in limited disruption of the intestinal microbiome composition, while possibly enhancing the presence of symbiotic bacteria, such as Bifidobacteria, that are commonly used in probiotics due to their reported benefits to improve intestinal barrier function and promote local anti-inflammatory cytokine production (23–25). Likewise, due to its limited systemic bioavailability, the side effect profile for rifaximin is quite favorable. We hypothesized that treatment with rifaximin could represent a unique strategy for reducing the generation of gut-derived cardiovascular toxins in CKD.

Our most important observation was that treatment with short-term rifaximin had no significant effect on circulating concentrations of the five gut-derived compounds of interest, along with several important proinflammatory cytokines (Figure 3, Table 2). This finding differs from observations in other studies that investigated the short-term effects of various antibiotic regimens on circulating concentrations of several of these compounds in both rodents and humans (18,19). For example, in an observational study examining changes in indoxyl sulfate and p-cresol sulfate in response to oral vancomycin therapy in patients with ESKD, serum concentrations of these compounds were significantly decreased after 28 days of therapy (19). Likewise, another investigation found that 1 week of therapy with broad-spectrum antibiotics substantially lowered serum TMAO concentrations after an oral phosphatidylcholine challenge in healthy adults (18). Although these investigations provide intriguing data to suggest the gut microbiota may be a potential therapeutic target for lowering cardiovascular risk, the primary drawback of these regimens is an inability to use them as chronic therapy, due to either the development of bacterial resistance or other potentially severe side effects.

Despite mounting evidence that suggests the gut microbiome may be an important therapeutic target for lowering cardiovascular risk in patients with CKD, a variety of strategies to improve gut health in CKD have been largely disappointing, or have led to only modest improvements in cardiovascular risk factors. Such interventions have included the use of adsorbents for binding bacterial metabolites in the gut lumen (38); prebiotics to provide beneficial nutrients (*i.e.*, short-chain fatty acids), which promote growth of more symbiotic bacterial populations (39); probiotics that directly introduce favorable bacterial populations to the intestinal environment (40); or synbiotics that combine prebiotics and probiotics into a single therapy (41–45). To date, these investigations have primarily been small clinical trials (<50 patients with CKD or ESKD) that have used biomarkers, rather than clinical end points, as the primary and secondary outcomes of interest. The one exception is a large, placebo-controlled trial investigating the effect of AST-120, an oral adsorbent, on kidney disease progression (38). This was a multinational trial that included >2000 study participants with late-stage CKD, and it was conducted over approximately 4 years. Disappointingly, this trial found AST-120 to demonstrate no benefit over placebo for CKD progression or the secondary outcomes, which included death. Additionally, because this study did not include measurements of gut-derived uremic toxins, the effect of AST-120 on these compounds remains undefined.

Multiple factors may have contributed to the inability of rifaximin to lower serum concentrations of gut-derived metabolites in our study. It is plausible that rifaximin could only affect these targets with chronic therapy or with much higher daily doses; however, on the basis of the previously mentioned literature regarding the efficacy of rifaximin in the treatment of other diseases, we suspect that the chosen duration of therapy and medication dosing were not major contributors to our negative findings. Recent evidence suggests certain bacterial populations have differing abilities to generate these uremic toxin precursors (46), so it seems

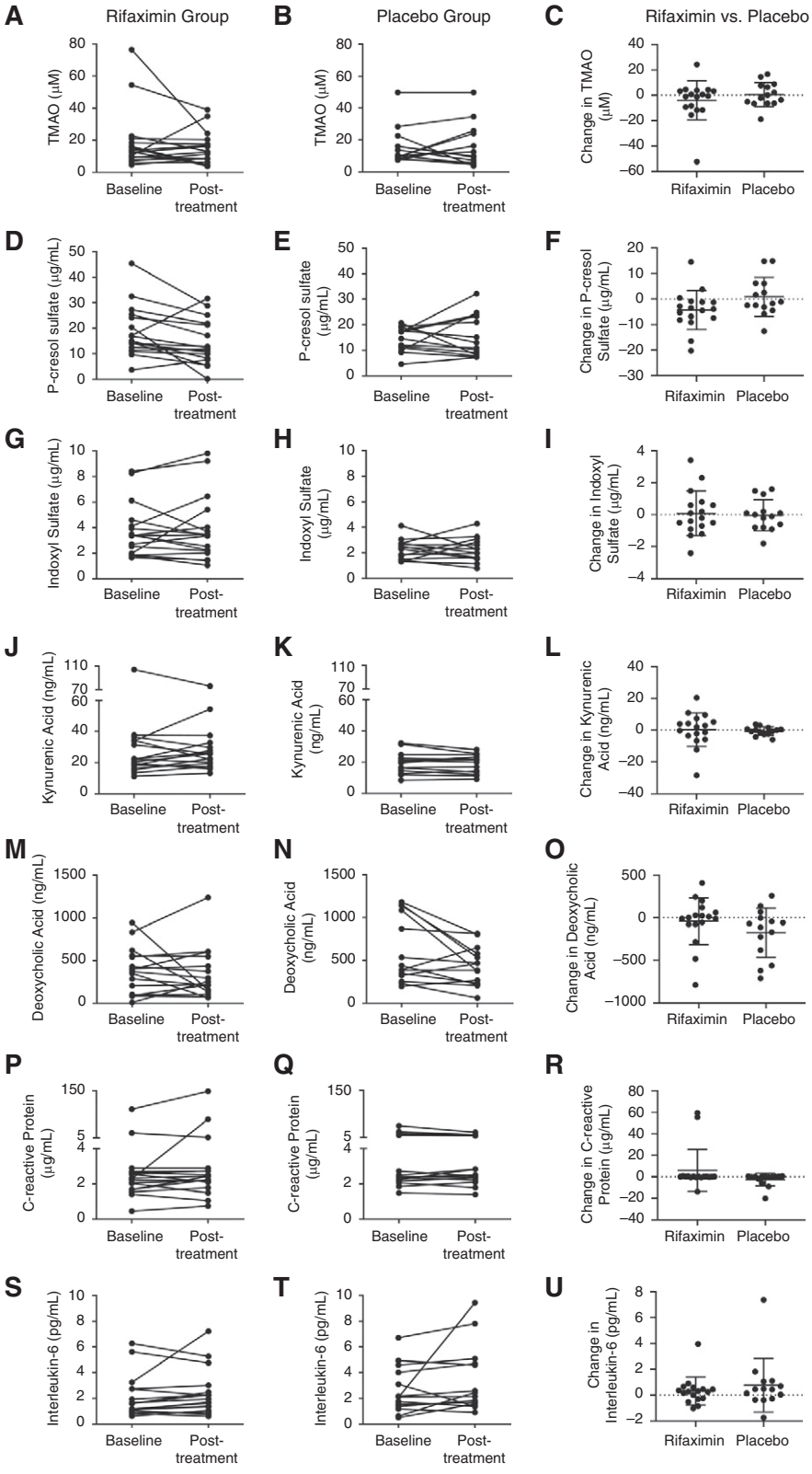

Figure 3. | **Rifaximin therapy fails to alter serum levels of gut-derived metabolites or inflammatory cytokines.** Pre- and post-treatment concentrations in individual study participants from the rifaximin group (first column) and placebo group (second column), along with a between-group comparison (far right column) of absolute changes for (A–C) trimethylamine *N*-oxide (TMAO), (D–F) p-cresol sulfate, (G–I) indoxyl sulfate, (J–L) kynurenic acid, (M–O) deoxycholic acid, (P–R) C-reactive protein, (S–U) and IL-6 (error bars represent mean±SD).

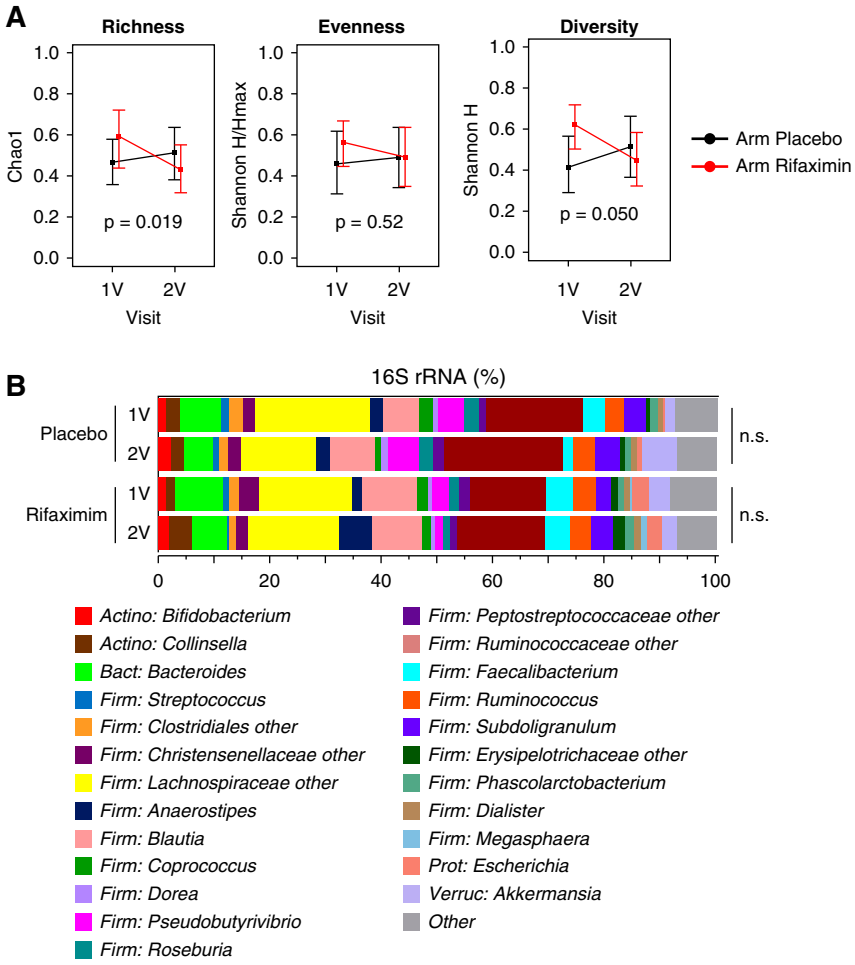

Figure 4. | **Rifaximin therapy reduces fecal bacterial richness and diversity.** (A) Changes in fecal bacterial richness, evenness, and diversity, stratified by treatment arm, assessed by a rank-based nonparametric test; error bars represent point estimates and confidence intervals for relative treatment effects. Average abundance of bacterial genera per group at each study visit depicted as percentage 16S rRNA for each genus relative to the total 16S rRNA count per sample. (B) Average relative abundance of predominant bacterial genera per group at each study visit. Differences in community composition (*i.e.*, β-diversity) after treatment were evaluated by the permutational multivariate ANOVA test. Changes in fecal bacterial richness, evenness, and diversity stratified by treatment arm (error bars represent mean±SD). 1V, baseline visit; 2V, post-therapy visit; Actino, Actinobacteria; Bact, Bacteroidetes; Firm, Firmicutes; Verr, Verrucomicrobia.

more likely that rifaximin lacks specificity for bacterial populations involved in the generation of our compounds of interest. It was not unexpected that rifaximin only resulted in subtle changes in bacterial stool populations, because this has been well documented (25,26). Lastly, it is plausible that rifaximin did lead to subtle reductions in the generation of the precursor metabolites of these gut-derived toxins; however, the downstream enzymatic reactions that form the final by-product may be upregulated in CKD. In support of this potential scenario, our group has previously observed rodents with CKD to exhibit increased hepatic flavin monooxygenase activity (47,48), the final enzyme responsible for the conversion of trimethylamine to TMAO. Likewise, alternative experiments, conducted in both rodents and humans, have suggested that intestinal dysbiosis in CKD may enhance the production of p-cresol sulfate and indoxyl sulfate from their amino acid precursors (49,50).

Our investigation has several important strengths and limitations. Notable strengths include the use of a placebo control and double blinding, a study cohort with comparable baseline demographics in many important categories (*i.e.*, age, race, body mass index, and baseline metabolite concentrations), thorough statistical analyses, the measurement of five separate bacterial metabolites using gold-standard methodology, and inclusion of data on changes in the composition of stool microbiota. Important limitations include the relatively short duration of therapy, a slightly skewed distribution of several baseline demographics between treatment arms (sex, eGFR, diabetes), a lack of knowledge of dietary patterns for study participants, and the use of biomarkers as primary and secondary outcomes.

In conclusion, although rifaximin therapy clearly has benefit in treating a variety of chronic diseases characterized by intestinal pathology, our current investigation found

Table 3. Change in the relative abundance of specific fecal bacteria taxa

Taxa[a]	P[b]	FDR[c]	Arm	Relative Abundance (%)[d]	
				Visit 1	Visit 2
Firm: *Clostridium*	0.0003	0.020	Placebo	0.115 (0.004–0.901)	0.232 (0.038–0.976)
			Rifaximin	0.218 (0.026–0.634)	0.003 (0.001–0.007)
Firm: *Turicibacter*	0.001	0.029	Placebo	0.014 (0.004–0.090)	0.097 (0.003–0.416)
			Rifaximin	0.003 (0.001–0.199)	0.000 (0.000–0.002)
Firm: *Anaerotruncus*	0.004	0.096	Placebo	0.307 (0.127–1.190)	0.358 (0.199–0.495)
			Rifaximin	0.306 (0.204–0.389)	0.141 (0.090–0.175)
Firm: *Streptococcus*	0.008	0.144	Placebo	0.156 (0.091–0.580)	0.384 (0.104–1.183)
			Rifaximin	0.324 (0.142–1.728)	0.229 (0.088–0.451)
Firm: *Anaerofustis*	0.01	0.145	Placebo	0.007 (0.004–0.029)	0.014 (0.010–0.022)
			Rifaximin	0.011 (0.006–0.016)	0.006 (0.001–0.014)
Firm: Christensenellaceae, other	0.01	0.145	Placebo	0.426 (0.277–1.666)	0.817 (0.182–1.959)
			Rifaximin	1.812 (0.321–7.799)	0.821 (0.103–2.888)
Firm: Family XIII, *Incertae sedis*, other	0.01	0.145	Placebo	0.328 (0.207–0.969)	0.537 (0.185–0.683)
			Rifaximin	0.563 (0.308–0.766)	0.200 (0.061–0.339)
Firm: *Eubacterium*	0.02	0.165	Placebo	0.000 (0.000–0.011)	0.000 (0.000–0.010)
			Rifaximin	0.000 (0.000–0.007)	0.000 (0.000–0.000)
Acti: *Actinomyces*	0.03	0.241	Placebo	0.023 (0.006–0.041)	0.024 (0.016–0.048)
			Rifaximin	0.036 (0.008–0.104)	0.016 (0.005–0.051)
Firm: Peptostreptococcaceae, other	0.05	0.377	Placebo	0.406 (0.085–2.520)	0.814 (0.186–2.316)
			Rifaximin	1.343 (0.212–3.441)	0.041 (0.008–1.453)

FDR, false discovery rate; Firm, Firmicutes; Acti, Actinobacteria.
[a]Genus-level taxa differing by treatment arm and time point. Taxa are listed in descending order of relative abundance. Names are prepended with phylum names. Families that could not be classified into individual genera are designated with "other."
[b]P values for nonparametric test comparing change in relative abundance over time between placebo and rifaximin. Only taxa with $P \leq 0.05$ are displayed.
[c]False discovery rate–corrected P values.
[d]Median relative abundances and interquartile ranges of taxa.

short-term rifaximin treatment had no effect on circulating concentrations of gut-derived cardiovascular toxins in patients with CKD. Despite these negative results, this study represents an important step to help guide the design of future investigations testing therapies that target the gut microbiota and intestinal pathology in CKD. This study also highlights the need for future investigations focused on better deciphering the complex interactions that exist been gut microbes, gut barrier integrity, kidney function, and cardiovascular pathology. Although these pathways continue to represent a promising target for lowering morbidity and mortality in this patient population, a more nuanced understanding of the interrelationships between these biologic systems will be necessary to design targeted therapies with a greater chance of successfully reducing cardiovascular pathology in CKD.

Disclosures

All authors have nothing to disclose.

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Author Contributions

M. Chonchol, D. Frank, A. Hoofnagle, C. Johnson, B. Kestenbaum, C. Kimber, J. Mahnken, M. Miyazaki, T. Nolin, J. Stubbs, A. Yu, and S. Zhang were responsible for methodology; D. Frank, C. Kimber, J. Mahnken, T. Nolin, J. Stubbs, and A. Yu conceptualized the study; D. Frank, C. Kimber, J. Mahnken, T. Nolin, J. Stubbs, and A. Yu provided supervision; D. Frank, A. Hoofnagle, D. Ir, C. Johnson, A. Jovanovich, B. Kestenbaum, C. Kimber, J. Mahnken, M. Miyazaki, T. Nolin, A. Prokopienko, C. Robertson, J. Stubbs, R. West, and S. Zhang were responsible for data curation; D. Frank, A. Hoofnagle, D. Ir, B. Kestenbaum, C. Kimber, A. Prokopienko, J. Mahnken, M. Miyazaki, T. Nolin, C. Robertson, and J. Stubbs were responsible for formal analysis; D. Frank, A. Jovanovich, B. Kestenbaum, C. Kimber, J. Mahnken, T. Nolin, J. Stubbs, and A. Yu reviewed and edited the manuscript; A. Hoofnagle, A. Jovanovich, B. Kestenbaum, M. Miyazaki, T. Nolin, J. Stubbs, R. West, and S. Zhang were responsible for validation; C. Johnson, C. Kimber, T. Nolin, J. Stubbs, A. Yu, and S. Zhang were responsible for investigation; C. Kimber, T. Nolin, and J. Stubbs wrote the original draft; C. Kimber, J. Stubbs, and A. Yu were responsible for project administration; J. Stubbs was responsible for funding acquisition and resources; and all authors approved the final version of the manuscript and agree to be accountable for all aspects of the work.

Data Sharing Statement

Deidentified data related to this study, including additional patient demographics, laboratory tests, and supporting clinical trial documents, will be available upon request from the corresponding author after manuscript publication.

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