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 ORIGINAL ARTICLE

Impact of Low-Dose Oral Minocycline (DFD-29) on Skin, Gastrointestinal, and Vaginal Microflora in Healthy Adults

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ABSTRACT

Background: DFD-29 (minocycline hydrochloride extended-release capsules, 40 mg) has shown significant therapeutic benefit vs placebo and doxycycline in treating moderate-to-severe rosacea. However, the impact of its use on skin, vaginal, and gastrointestinal microbiota is unknown.

Methods: In this multicenter, randomized, double-blind, placebo-controlled trial, 60 healthy adults were randomized in a 2:1 ratio to receive either DFD-29 (40 mg) orally or a matching placebo once daily for 16 weeks. Microbiological samples were collected from the skin (forehead), vagina, and stool at baseline and weeks 4, 8, and 16 to evaluate changes in normal microbiota species (via culture and 16S rRNA sequencing), in the MIC90 of selected colonized microbial species, and in opportunistic microbiota with DFD-29 vs placebo. Safety was evaluated via analysis of adverse events, vital signs, and laboratory tests.

Results: Thirty-eight adults assigned to DFD-29 and 19 adults assigned to placebo were included in the microbiota evaluable population. There were no significant differences detected in the abundance of microbial species in the skin, stool, or vagina from baseline to week 16 between the DFD-29 and placebo groups. No significant differences were detected in resistance to minocycline between DFD-29 and placebo. There were also no significant differences in the presence of opportunistic microbiota at any time point. No significant safety issues were reported.

Conclusion: Administration of DFD-29 for 16 weeks had no detectable effects on skin, GI tract, or vaginal microflora and was well tolerated in healthy adults, reinforcing its potential as a therapeutic option in moderate-to-severe rosacea.

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INTRODUCTION

Rosacea is a chronic inflammatory facial skin disease that affects 5% to 10% of the population and can adversely affect quality of life.[1,2] Common signs and symptoms of rosacea include skin erythema, the development of erythematous papules and pustules, flushing, telangiectasia, phymatous changes, and ocular manifestations.[3,4] While its pathogenesis is not fully understood, it is believed to be multifaceted and involves immune and inflammatory dysregulation, neurovascular dysregulation, microbiome dysbiosis, and genetics.[5-10] Systemic antibiotics, such as minocycline and doxycycline, are frequently used to control rosacea symptoms.

Minocycline is a chemically modified, second-generation, broad-spectrum tetracycline that effectively targets a wide range of Gram-positive and Gram-negative bacteria and atypical organisms,[11] preventing bacterial growth by inhibiting protein synthesis.[12] It also exhibits potent anti-inflammatory properties.[11]

A low-dose formulation of minocycline hydrochloride (HCl), DFD-29, has shown superior therapeutic benefit vs placebo and doxycycline in treating rosacea and has recently been approved for this indication by the US Food and Drug Administration under the brand name of Emrosi™ (minocycline hydrochloride).[13-15] However, the impact of DFD-29 on skin, gastrointestinal (GI), and vaginal microbiota is unknown. This study evaluated whether treatment with DFD-29 results in shifts in the normal microbiota of the skin, GI tract, or vagina, the development of resistance to minocycline, and the appearance of or increase in opportunistic microbiota (yeast or opportunistic bacteria).

MATERIALS AND METHODS

This multicenter, randomized, double-blinded, placebo-controlled, parallel-group Phase 1 trial (NCT05597462) compared the impact of DFD-29 (minocycline hydrochloride extended-release capsules; Emrosi, Journey Medical Corporation) and matching placebo on skin, GI, or vaginal microflora in 60 healthy adults (30 males and 30 females) aged 18 to 65 years (Figure 1). The study was conducted between September 28, 2022, and February 24, 2023, at 2 sites in El Paso, Texas, and Las Cruces, New Mexico. Both studies followed Good Clinical Practice, US local laws and regulations, and the Declaration of Helsinki. Institutional review boards at each study center approved the protocol and consent forms, and written informed consent was obtained from all participants.

Subjects were eligible for the trial if they were aged ≥18 and ≤65 years, agreed to avoid using another antibiotic during the 16-week treatment period, were not pregnant or lactating, agreed to use an acceptable method of birth control (women of childbearing potential and all male subjects), and were in good health and free from any clinically significant disease that may have interfered with the evaluation of microbiota or the administration or absorption of study drugs.

Individuals were excluded if they had a history of vaginitis within 3 months of randomization; had used systemic non-tetracycline antibiotics within 6 weeks or systemic tetracycline antibiotics within 3 months; had been treated with systemic retinoids or therapeutic vitamin A supplements within 180 days; or used topical retinoids, antibiotics, corticosteroids, anti-mycotic agents, azelaic acid, or ivermectin on the face within 30 days; or were unable or unwilling to provide fecal/stool samples or self-collect vaginal swabs.

Subjects were randomly assigned in a 2:1 ratio to DFD-29 40 mg or matching placebo capsules once daily orally for 16 weeks (113 days). Randomization was stratified by gender, so that approximately an equal number of male and female subjects were enrolled within each treatment group, using a randomization schedule generated by an independent statistician. The study consisted of a screening period (day -30 to day -1) and a treatment period (day 1 to day 113) and included 5 visits.

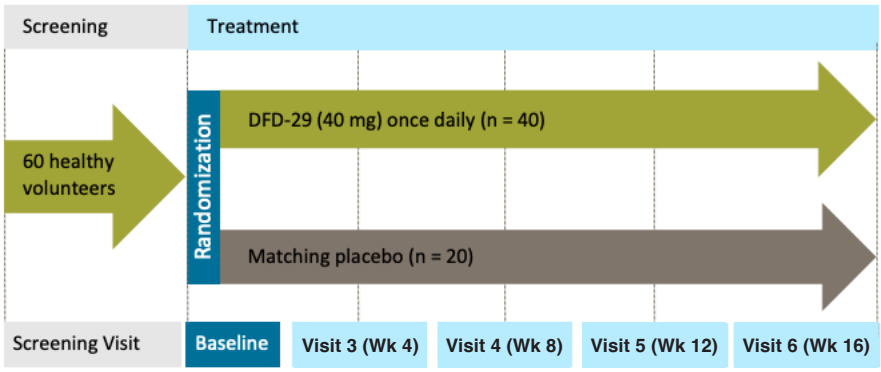
Subjects were instructed to take oral DFD-29 or a placebo once daily at a fixed time of day (preferably in the morning after an overnight fast) with 240 mL (1 glass) of water and were advised to avoid consuming substances that could potentially interfere with minocycline absorption 1.5 hours before to 3.0 hours after taking the study drug. Both DFD-29 and matching placebo capsules were provided by Dr. Reddy's Laboratories Ltd, in Telangana, India. All investigators, study staff, and sponsor staff were blinded to study drugs until study closure.

At each visit, microbiologic assessments of skin swabs, stool samples, and vaginal swabs were performed to evaluate the impact of DFD-29 or placebo on the microbiome of the skin, GI tract (through the stool), and the vagina. Trained staff collected skin swab samples from 5 different forehead regions using 3 swabs designated for bacterial 16S rRNA taxonomic identification and 2 swabs designated for microbial culture. Subjects collected stool samples within 4 hours of each scheduled visit. Female subjects were instructed to self-collect vaginal samples at a private location at the study site by inserting and briefly swirling 5 swabs from different interior regions of the vaginal surface, starting with the deepest region.

Swabs were then placed into culture media tubes (Cary-Blair collection tubes) for shipment within 12 hours to the central laboratory (Ecco Labs). Samples were then sowed and incubated for 48 hours in standardized culture media, and pathogenic microbial agents and normal culturable microflora were identified.

The minimum inhibitory concentration (MIC) against minocycline in the isolated strains was determined using the Epsilometer (E-Test).[16] The 2022 Clinical and Laboratory Standards Institute (CLSI) M100 database was then used to generate a list of species sensitive to minocycline based on the MIC categorization of sensitivity. The minocycline MIC_{90} was determined using

FIGURE 1. Study design.



Wk; week.

a gradient diffusion method specified in the CLSI guidelines. Sensitive (S) species included those with MIC_{90} of ≤4 µg/mL; species with indeterminant sensitivity (I) had a MIC_{90} of 4.1 to 15.9 µg/mL, and resistant species (R) had a minocycline MIC_{90} of ≥16 µg/mL. The top 10% most frequent organisms at baseline were analyzed.

To complete the microbial gene analysis, the pooled swabs for bacterial 16S rRNA taxonomic identification were placed into Norgen Biotek DNA collection and preservation tubes, frozen at -70 °C within 12 hours, and shipped under dry ice to the central laboratory (Creative Biogen) in batches for long-chain 16S sequencing.

The primary outcome of the study was the shift in culturable levels of microbial species colonized from skin, the GI tract, and vaginal swabs from baseline to week 16 in the DFD-29 group compared to the placebo group. Changes in the expression levels of the predominant microbial operational taxonomic units (OTUs) detected from 16S ribosomal RNA (rRNA) gene analysis at each site from baseline to week 16 in the DFD-29 the placebo group were the supportive analysis. Target sequences were amplified by polymerase chain reaction, and their products were purified, quantified, and homogenized to generate SMRT bell libraries. Qualified libraries were processed for sequencing on the PacBio Sequel platform and converted into circular consensus sequencing (CCS) files by SMRTlink. Effective CCS were clustered into OTUs/amplicon sequence variants (ASVs) and processed following feature analysis.

The Shannon Diversity Index was computed for all samples to determine the abundance and distribution of abundance of microbial species at each site. Changes in antibiotic resistance (MIC_{90}) and appearance of opportunistic microbiota (Candida albicans or bacteria) from baseline to week 16 in the DFD-29 group vs placebo were also evaluated. Safety was assessed by evaluation of adverse events (AEs) at each visit.

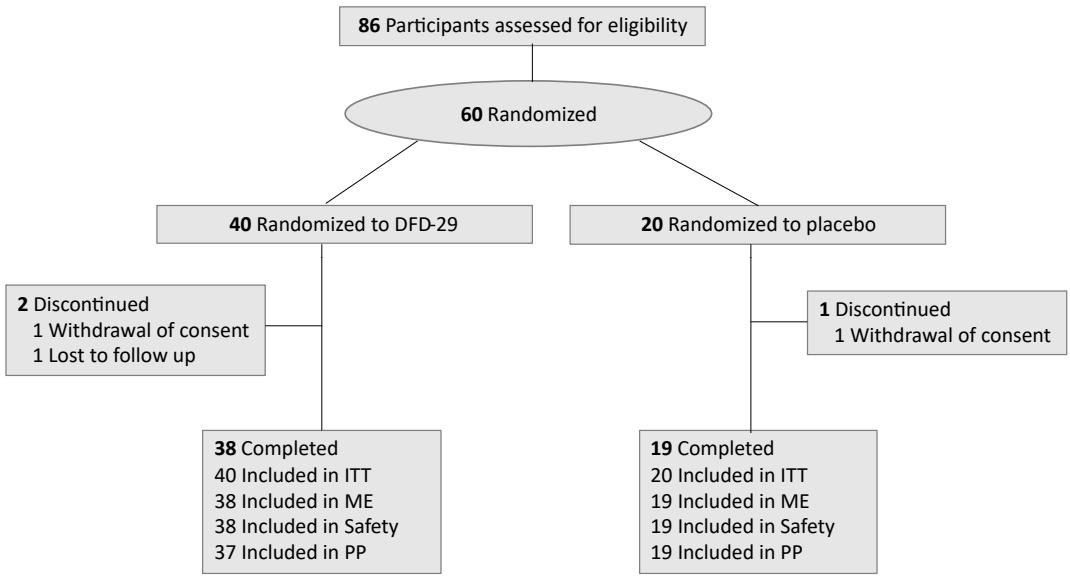
No formal statistical sample size estimation was performed for this Phase 1 study. The Safety population included all randomized subjects who had received at least one dose of study medication and had at least one post-baseline safety assessment. The ITT population included all randomized subjects. The microbiota evaluable (ME) population, the primary population for microbiologic assessments, included all intention-to-treat (ITT) subjects who provided microbiology samples for skin, stool, or vagina at both baseline and week 16.

Changes in the percentage of subjects with cultured microbiota at each site were evaluated using descriptive statistics. Treatment groups were compared at weeks 4, 8, and 16 using Fisher's exact tests. Comparisons in changes in the relative abundance of microorganisms, Shannon Diversity Indices, and MIC values were summarized by site and calculated using Wilcoxon rank sum tests. Microbiota species appearing at week 16 and not baseline were considered opportunistic based on growth density and if they were from a genus known in the literature to be opportunistic or pathogenic. The proportions of subjects with at least 1 opportunistic microbiota that met the above criteria were assessed and compared between groups using Fisher's exact tests.

RESULTS

Among 86 subjects screened, 60 were randomized, and 57 completed the study (Figure 2). Thirty-eight adults assigned to DFD-29 and 19 adults assigned to placebo were included in the

FIGURE 2. Subject disposition.



ITT; intention-to-treat, ME; microbiota evaluable. PP; per protocol.

ME population. Demographic and baseline characteristics of the study subjects were comparable between the groups (Table 1). The mean age of the subjects was 43.4 years (11.9 SD). All subjects were White, 50.9% were female (n = 29), and 94.7% (n = 54) were Hispanic or Latino. Mean BMI was 30.8 kg/m^2 (6.5 SD).

Table 2 presents the number and proportion of individuals with the 10% most prevalent microorganisms grown from the swabs or fecal samples after use of either DFD-29 or placebo. There was no clinically relevant impact on levels of cultured microbiota across the skin, gastrointestinal tract, or vagina with DFD-29 treatment when compared to placebo at baseline, week 4, week 8, or week 16. Although a high degree of variability was noted in the cultured microbiota from individual subjects over the treatment period, no individual subject showed a clinically meaningful increase or decrease in any microbial species.

MIC_{90} values for minocycline in the DFD-29 and placebo groups were similar in pre-identified microbiota over time (Table 3). Among subjects with the identified genera at baseline, changes in resistance patterns did not differ between the DFD-29 and placebo groups. There was no evidence that MIC_{90} values at any of the body sites indicated a change from minocycline sensitivity to minocycline resistance.

Figure 3 shows the number of subjects who had at least 1 cultured opportunistic microorganism present at baseline and at week 16. Only *Staphylococcus* and *Enterococcus faecalis* is were identified as preselected candidates for opportunism. The number of subjects with these bacteria was low, and no subject had heavy culture growth at any time. A few cultures from the skin and vagina had growth of *Candida* spp at baseline, but the number of these subjects was similar between groups and did not increase at week 16 in either group. There was no indication that pathogenic growth resulted in AEs or in physical signs or symptoms.

The top 10 most relatively abundant OTU/family/genus identified at baseline by 16S rRNA sequencing by body region in the supportive OTU analysis are summarized in Table 4. While there were variable changes in the relative abundance of OTUs between treatments from baseline to 16 weeks, these differences were not clinically meaningful. Although statistically significant differences in the abundance of *Lactobacillus*, *Streptococcus*, and *Prevotella* species from vaginal swabs at week 16 were seen in the DFD-29 and placebo groups, these changes were not associated with any shifts in the cultured microbiota and did not result in any clinical signs or symptoms. For the supportive analysis of microbial diversity, changes in the Shannon Diversity Index from baseline to week 16 were neither clinically relevant nor statistically significant for any of the identified genera.

Overall, 3 subjects (5.3%) had at least 1 treatment-emergent AE (TEAE), including 2 subjects (5.3%) in the DFD-29 group and 1 (5.3%) in the placebo group. The two TEAEs in the DFD-29 group were UTIs, diagnosed in two female subjects based on the presence of red blood cells in the urine. There were no clinical signs or symptoms of UTI, and cultures were not performed to confirm UTI. One subject in the placebo group reported a headache. No severe TEAEs were reported, and no TEAEs led to treatment discontinuation.

TABLE 1.

Demographics and baseline characteristics*

Characteristic	DFD-29 (n = 38)	Placebo (n = 19)	Total (N = 57)
Age, y			
Mean (SD)	42.4 (11.9)	45.3 (12.0)	43.4 (11.9)
Median (range)	41.0 (20-64)	48.0 (24-63)	42.0 (20-64)
Sex, n (%)			
Male	18 (47.4)	10 (52.6)	28 (49.1)
Female	20 (52.6)	9 (47.4)	29 (50.9)
Race, n (%)			
White	38 (100.0)	19 (100.0)	57 (100.0)
Ethnicity, n (%)			
Hispanic or Latino	36 (94.7)	18 (94.7)	54 (94.7)
Not Hispanic or Latino	2 (5.3)	1 (5.3)	3 (5.3)
Mean BMI, kg/m^2 (SD)	29.7 (6.0)	33.1 (7.1)	30.8 (6.5)

*Safety population.
BMI; body mass index, SD; standard deviation.

TABLE 2.

Presence of Culturable Microbiota in ME population. Data Presented as n (%).				
Normal (culturable) flora	**Assessment**	**DFD-29 (n = 38)**	**Placebo (n = 19)**	***P* value**
Skin				
Coagulase-negative *Staphylococcus*	Baseline	16 (42.1)	3 (15.8)	0.518
	Week 16	19 (50.0)	12 (63.2)	
Propionibacterium acnes	Baseline	10 (26.3)	4 (21.1)	0.372
	Week 16	4 (10.5)	4 (21.1)	
Staphylococcus epidermidis	Baseline	8 (21.1)	4 (21.1)	1.000
	Week 16	0 (0)	0 (0)	
Staphylococcus hominis	Baseline	3 (7.9)	3 (15.8)	1.000
	Week 16	0 (0)	0 (0)	
Viridans group *Streptococcus spp*	Baseline	6 (15.8)	4 (21.1)	1.000
	Week 16	2 (5.3)	0 (0)	
Stool				
Citrobacter freundii	Baseline	8 (21.1)	1 (5.3)	0.028
	Week 16	2 (5.3)	4 (21.1)	
Coagulase-negative *Staphylococcus*	Baseline	2 (5.3)	6 (31.6)	0.274
	Week 16	4 (10.5)	0 (0)	
Enterobacter aerogenes	Baseline	11 (28.9)	3 (15.8)	0.433
	Week 16	4 (10.5)	5 (26.3)	
Enterobacter cloacae	Baseline	6 (15.8)	2 (10.5)	1.000
	Week 16	0 (0)	0 (0)	
Enterococcus spp	Baseline	22 (57.9)	9 (47.4)	0.537
	Week 16	26 (68.4)	15 (78.9)	
Escherichia coli	Baseline	34 (89.5)	18 (94.7)	1.000
	Week 16	35 (92.1)	18 (94.7)	
Klebsiella oxytoca	Baseline	8 (21.1)	0	0.158
	Week 16	3 (7.9)	4 (21.1)	
Klebsiella pneumoniae	Baseline	15 (39.5)	9 (47.4)	0.512
	Week 16	12 (31.6)	4 (21.1)	
Proteus mirabilis	Baseline	10 (26.3)	4 (21.1)	1.000
	Week 16	5 (13.2)	3 (15.8)	
Pseudomonas aeruginosa	Baseline	6 (15.8)	3 (15.8)	0.495
	Week 16	11 (28.9)	7 (36.8)	
Vagina*				
Escherichia coli	Baseline	13 (34.2)	7 (36.8)	0.686
	Week 16	10 (26.3)	3 (15.8)	
Lactobacillus spp	Baseline	11 (28.9)	5 (26.3)	0.250
	Week 16	9 (23.7)	2 (10.5)	
	Baseline	10 (26.3)	2 (10.5)	
Coagulase-negative *Staphylococcus*	Week 16	11 (28.9)	4 (21.1)	1.000
Corynebacterium spp	Baseline	7 (18.4)	2 (10.5)	1.000
	Week 16	3 (7.9)	1 (5.3)	
Enterococcus spp	Baseline	6 (15.8)	3 (15.8)	0.153
	Week 16	15 (39.5)	9 (47.4)	
Klebsiella pneumoniae	Baseline	6 (15.8)	3 (15.8)	1.000
	Week 16	3 (7.9)	1 (5.3)	

*Percentages are calculated based on the total population and not the number of subjects who underwent vaginal assessments (n = 20 for DFD-29 and n = 9 for placebo).

TABLE 3.

Change in MIC_{90} to Minocycline Values From Baseline to Week 16 in Cultivated Microbiota*					
	DFD-29 (n = 38)		Placebo (n = 19)		*P* value
	n	Mean change in MIC (SD)	n	Mean change in MIC (SD)	
Skin					
Coagulase-negative *Staphylococcus*	8	0.71 (4.22)	2	2.49 (0.93)	0.695
Propionibacterium acnes	2	2.55 (0.43)	1	1.94 (0.00)	0.540
Staphylococcus aureus	0[a]	0	1	-8.40 (0.00)	N/A
Stool					
Enterobacter aerogenes	0[a]	0	1	3.00 (0.00)	N/A
Enterococcus spp	15	-17.4 (21.6)	7	-1.57 (4.69)	0.091
Escherichia coli	31	-1.55 (5.51)	17	-6.78 (16.6)	0.605
Klebsiella pneumoniae	7	-3.01 (7.84)	7	-0.98 (2.74)	0.925
Vagina					
Coagulase-negative *Staphylococcus*	6	-17.4 (23.6)	0[a]	0	N/A
Corynebacterium spp	1	0	0[a]	0	N/A
Escherichia coli	6	-2.17 (5.43)	6	-7.63 (4.02)	0.245
Lactobacillus spp	3	1.04 (0.57)	0[a]	0	N/A

*Data from the ME population are presented.
MIC_{90}, minimal inhibitory concentration for minocycline; SD, standard deviation.

FIGURE 3. Subjects with opportunistic organisms present at week 16 but not present at baseline in the ME population. No significant between-group differences were observed.

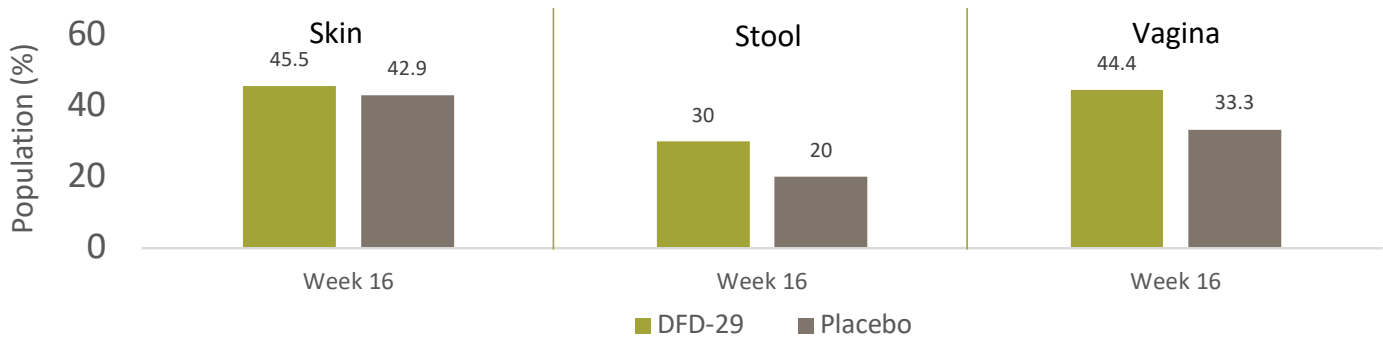


TABLE 4.

Taxonomic Microbiome Summary of Mean Changes (SD) in Abundance Values in the ME Population.

Genus	DFD-29 (n = 38)				Placebo (n = 17)				*P* value
	n	Baseline	Week 16	Change	n	Baseline	Week 16	Change	
Skin									
Prevotella 9	38	0.144 (0.199)	0.144 (0.207)	0.00 (0.148)	17	0.176 (0.207)	0.120 (0.233)	-0.057 (0.208)	0.299
Escherichia shigella	38	0.018 (0.037)	0.008 (0.023)	-0.010 (0.041)	17	0.043 (0.136)	0.018 (0.050)	-0.024 (0.134)	0.669
Bacteroides	38	0.241 (0.146)	0.307 (0.204)	0.067 (0.141)	17	0.227 (0.172)	0.252 (0.172)	0.025 (0.143)	0.367
Faecalibacterium	38	0.111 (0.092)	0.065 (0.046)	-0.045 (0.091)	17	0.088 (0.050)	0.085 (0.069)	-0.002 (0.084)	0.295
Ruminococcus gnavus group	38	0.011 (0.061)	0.005 (0.012)	-0.006 (0.058)	17	0.005 (0.011)	0.002 (0.005)	-0.003 (0.008)	0.337
Fusobacterium	38	0.021 (0.069)	0.018 (0.069)	-0.003 (0.089)	17	0.009 (0.034)	0.000 (0.000)	-0.009 (0.034)	0.834
Akkermansia	38	0.028 (0.065)	0.028 (0.093)	0.000 (0.117)	17	0.035 (0.058)	0.036 (0.097)	0.000 (0.113)	0.935
Prevotella	38	0.004 (0.010)	0.016 (0.071)	0.013 (0.070)	17	0.019 (0.052)	0.043 (0.134)	0.024 (0.122)	0.978
Klebsiella	27	0.012 (0.038)	0.001 (0.003)	-0.012 (0.038)	11	0.007 (0.012)	0.001 (0.001)	-0.006 (0.011)	0.628
Megamonas	11	0.001 (0.004)	0.001 (0.004)	0.000 (0.000)	6	0.025 (0.059)	0.016 (0.039)	-0.009 (0.020)	0.378
Stool									
Lactobacillus	38	0.315 (0.258)	0.028 (0.062)	-0.287 (0.270)	18	0.269 (0.237)	0.020 (0.036)	-0.250 (0.247)	0.630
Akkermansia	38	0.004 (0.005)	0.006 (0.018)	0.002 (0.020)	18	0.030 (0.108)	0.006 (0.016)	-0.024 (0.110)	0.951
Muribaculaceae unclassified	38	0.035 (0.042)	0.074 (0.054)	0.039 (0.068)	18	0.044 (0.060)	0.066 (0.057)	0.022 (0.088)	0.415
Prevotella	38	0.089 (0.062)	0.006 (0.012)	-0.083 (0.063)	18	0.088 (0.066)	0.030 (0.068)	-0.058 (0.106)	0.425
Gardnerella	38	0.040 (0.043)	0.005 (0.015)	-0.035 (0.047)	18	0.041 (0.054)	0.002 (0.003)	-0.039 (0.054)	0.605
Bacteroides	38	0.017 (0.032)	0.069 (0.075)	0.052 (0.084)	18	0.012 (0.009)	0.084 (0.058)	0.072 (0.056)	0.069
Dialister	38	0.018 (0.027)	0.003 (0.010)	-0.014 (0.025)	18	0.018 (0.026)	0.010 (0.029)	-0.008 (0.041)	0.732
Megasphaera	24	0.024 (0.022)	0.001 (0.001)	-0.023 (0.022)	13	0.018 (0.024)	0.002 (0.003)	-0.016 (0.025)	0.067
Lachnospiraceae NK4A136 group	38	0.010 (0.013)	0.030 (0.019)	0.020 (0.022)	18	0.012 (0.022)	0.027 (0.019)	0.015 (0.027)	0.630
Unclassified bacteria	34	0.012 (0.017)	0.002 (0.004)	-0.010 (0.018)	14	0.016 (0.027)	0.002 (0.003)	-0.014 (0.027)	0.725
Vagina									
Lactobacillus	19	0.497 (0.310)	0.290 (0.0386)	-0.208 (0.326)	8	0.367 (0.274)	0.523 (0.404)	0.155 (0.230)	0.007
Streptococcus	19	0.008 (0.014)	0.039 (0.134)	0.031 (0.124)	8	0.121 (0.305)	0.002 (0.002)	-0.120 (0.305)	0.002
Prevotella	19	0.149 (0.108)	0.180 (0.170)	0.031 (0.167)	8	0.132 (0.075)	0.049 (0.090)	-0.083 (0.150)	0.024
Haemophilus	19	0.015 (0.057)	0.013 (0.055)	-0.002 (0.005)	8	0.012 (0.025)	0.000 (0.000)	-0.012 (0.025)	0.506
Gardnerella	19	0.060 (0.067)	0.063 (0.115)	0.003 (0.094)	8	0.040 (0.041)	0.030 (0.059)	-0.010 (0.082)	0.474
Akkermansia	19	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	8	0.037 (0.077)	0.001 (0.002)	-0.037 (0.078)	0.107
Campylobacter	19	0.022 (0.048)	0.030 (0.061)	0.008 (0.028)	8	0.012 (0.015)	0.019 (0.047)	0.007 (0.054)	0.094
Sneathia	19	0.024 (0.045)	0.024 (0.066)	-0.001 (0.065)	7	0.011 (0.015)	0.000 (0.001)	-0.011 (0.016)	1.000
Fastidiosipila	19	0.028 (0.044)	0.006 (0.016)	-0.022 (0.039)	8	0.021 (0.036)	0.001 (0.002)	-0.020 (0.037)	0.770
Bacteroides	19	0.006 (0.017)	0.014 (0.051)	0.008 (0.051)	8	0.045 (0.062)	0.042 (0.064)	-0.003 (0.108)	0.811

SD, standard deviation

DISCUSSION

Systemic antibiotics are a mainstay for the treatment of moderate-to-severe rosacea.[17,18] Theoretically, the use of such broad-spectrum antibiotics for long durations could exert selective pressure on microorganisms, which could lead to resistance. The use of broad-spectrum antibiotics could impact the composition, diversity, and equilibrium of the microbiota, with the potential to lead to long-term dysbiosis if used at higher antimicrobial doses.[19,20]

For years, a modified-release formulation of doxycycline (40 mg once daily) has been the only oral tetracycline FDA-approved for the treatment of inflammatory lesions of rosacea.[21] However, oral minocycline has been used off-label to treat this condition for years, usually at a dose of 100 mg/day.[11,22]

DFD-29 provides the lowest approved dose of minocycline and a systemic minocycline exposure far lower than that seen with antimicrobial doses of minocycline. A previous pharmacokinetic study of DFD-29 reported a steady state C_{max} of ~300 ng/mL, which is significantly lower than the known MIC of 1000 ng/mL for minocycline.[15]

The results from this study confirm these findings and indicate that administration of DFD-29 for 16 weeks had no detectable effects on microbiota in the skin, GI tract, or vagina. DFD-29 was not associated with the development of resistance to minocycline in the microbiota at any site studied. DFD-29 also showed no significant increase in the frequency of opportunistic bacteria during or after treatment and had no clinically meaningful impact on the relative levels of bacterial OTUs as determined by 16S rRNA sequencing. Overall, use of DFD-29 for 16 weeks was generally safe and well tolerated in healthy adults.

There are few data on the impact of lower doses of minocycline or doxycycline on GI, skin, or vaginal microflora, although doxycycline 40 mg is considered a subantimicrobial dose.[23,24] A small trial of 4 women receiving 100 mg oral minocycline reported a 1.4-fold reduction in the mean relative abundance of *Cutibacterium acnes* in the skin microbiota at week 4 of treatment.[25] Decreases in the relative abundance of *Cutibacterium, Corynebacterium, Prevotella, Lactobacillus*, and *Porphyromonas* were also observed.

In contrast, increases in the relative abundance of *Streptococcus, Chryseobacterium, Finegoldia, Pseudomonas, Erwinia, Actinobacillus*, and *Micrococcus* were seen with minocycline, demonstrating that higher doses of oral minocycline can alter the skin's microbiome. Similarly, another study evaluating the effects of 100 mg oral minocycline given twice daily in 8 patients with acne reported significant changes in gut and skin microbiota following 4 weeks of treatment, including reductions in *Lactobacillus salivarius* (P=0.001), *Bifidobacterium adolescentis* (P=0.002), *Bifidobacterium pseudolongum* (P=0.010), and *Bifidobacterium breve* (P=0.042) in the gut, increases in *Bifidobacterium longum* (P=0.028) and *Leuconostoc mesenteroides* (P=0.029) in the skin, and reductions in *Staphylococcus epidermidis* (P=0.009) and *Prevotella nigrescens* (P=0.028) in the skin.[26]

Results from the present study support evidence from randomized, double-blind, placebo- and active-controlled studies of DFD-29 indicating its efficacy and tolerability in the treatment of moderate-to-severe rosacea.[14,15] In two recent phase 3 trials, DFD-29 (40 mg/d) demonstrated superior efficacy in the proportion of subjects achieving Investigator's Global Assessment (IGA) success vs placebo and doxycycline 40 mg/d. DFD-29 also showed superior efficacy in reducing inflammatory lesions vs both comparators and improved Clinician's Erythema Assessment vs placebo.[14] This sub-antimicrobial formulation of minocycline, with its superior efficacy vs doxycycline 40 mg/d, may provide significant advantages in rosacea management.[15]

In the current study, DFD-29 had no significant impact on the microbiota of the skin, GI, or vaginal tract and thus may be suitable for longer-term use compared to standard doses of minocycline. The results from this study are particularly clinically relevant given its use of the latest available microbiological techniques to assess the impact on normal and pathogenic microorganisms.

CONCLUSION

In conclusion, administration of DFD-29 for 16 weeks had no detectable effects on microbiota in the skin, GI tract, or vagina. Overall, the use of DFD-29 for 16 weeks was generally safe and well tolerated in healthy adults and may offer a useful option for patients with moderate-to-severe rosacea.

DISCLOSURES

Richard L. Gallo MD PhD is a consultant for Journey Medical Corporation. Hilary Baldwin MD is a consultant for and has received honoraria from Almirall, Arcutis, Beiersdorf, Cutera, Dermavant, Galderma, Journey, Kenvue, La Roche-Posay, l'Oreal, Nutrafol, Ortho, Sun, and Tarsus. Julie Harper MD is a consultant for and has received honoraria from Almirall, Arcutis, Beiersdorf, Bioderma, Bubble, Cutera, Galderma, Journey Medical Corporation, L'Oreal, Nutrafol, Ortho, and Sun. Srinivas Sidgiddi MD is an employee of Journey Medical Corporation. Neal Bhatia MD reports grants and/or research funding from AbbVie, Amgen, Arcutis, Bristol Myers Squibb, Boehringer Ingelheim, Dr. Reddy's Laboratories, DUSA Pharmaceuticals, Inc., LEO Pharma US, Mindera, Nutrafol, Pfizer, Sanofi/Regeneron, Soligenix, Sun Pharmaceutical Industries, Verrica Pharmaceuticals, and Zerigo Health, and personal fees from Almirall, Beiersdorf, Biofrontera AG, Castle Biosciences, Dermavant Sciences, Ferndale Laboratories, Galderma Laboratories, ISDIN, Johnson and Johnson Consumer Products Company, La Roche-Posay Laboratorie Pharmaceutique, Ortho Dermatologics, Practical Dermatology,

Sanofi/Regeneron, and Sun Pharmaceutical Industries. John A. McLane PhD is an employee of Catawba Research and reports no other conflicts of interest.

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REFERENCES

1. Gether L, Overgaard LK, Egeberg A, et al. Incidence and prevalence of rosacea: a systematic review and meta-analysis. *Br J Dermatol.* 2018;179(2):282-289.
2. Tan J, Schöfer H, Araviiskaia E, et al. Prevalence of rosacea in the general population of Germany and Russia - The RISE study. *J Eur Acad Dermatol Venereol.* 2016;30(3):428-434.
3. Gallo RL, Granstein RD, Kang S, et al. Standard classification and pathophysiology of rosacea: The 2017 update by the National Rosacea Society Expert Committee. *J Am Acad Dermatol.* 2018;78(1):148-155.
4. Tan J, Berg M, Gallo RL, et al. Applying the phenotype approach for rosacea to practice and research. *Br J Dermatol.* 2018;179(3):741-746.
5. Akamatsu H, Asada M, Komura J, et al. Effect of doxycycline on the generation of reactive oxygen species: a possible mechanism of action of acne therapy with doxycycline. *Acta Derm Venereol.* 1992;72(3):178-179.
6. Cazalis J, Bodet C, Gagnon G, et al. Doxycycline reduces lipopolysaccharide-induced inflammatory mediator secretion in macrophage and ex vivo human whole blood models. *J Periodontol.* 2008;79(9):1762-1768.
7. Griffin MO, Ceballos G, Villarreal FJ. Tetracycline compounds with non-antimicrobial organ protective properties: possible mechanisms of action. *Pharmacol Res.* 2011;63(2):102-107.
8. Hoyt JC, Ballering J, Numanami H, et al. Doxycycline modulates nitric oxide production in murine lung epithelial cells. *J Immunol.* 2006;176(1):567-572.
9. Ishikawa C, Tsuda T, Konishi H, et al. Tetracyclines modulate protease-activated receptor 2-mediated proinflammatory reactions in epidermal keratinocytes. Antimicrob Agents Chemother. 2009;53(5):1760-1765.
10. Lee JJ, Chien AL. Rosacea in older adults and pharmacologic treatments. *Drugs Aging.* 2024;41(5):407-421.
11. Martins AM, Marto JM, Johnson JL, et al. A review of systemic minocycline side effects and topical minocycline as a safer alternative for treating acne and rosacea. *Antibiotics (Basel).* 2021;10(7).
12. Nazarian S, Akhondi H. Minocycline. In: *StatPearls.* Treasure Island (FL) ineligible companies. Disclosure: Hossein Akhondi declares no relevant financial relationships with ineligible companies.: StatPearls Publishing Copyright © 2024, StatPearls Publishing LLC.; 2024.
13. Emrosi™ (minocycline hydrochloride) prescribing information. In. Scottsdale, AZ: Journey Medical Corporation; 2024.
14. Bhatia N, Del Rosso J, Stein Gold L, et al. Efficacy, safety, and tolerability of oral DFD-29, a low-dose formulation of minocycline, in rosacea: two phase 3 randomized clinical trials. *JAMA Dermatol.* 2025.
15. Tsianakas A, Pieber T, Baldwin H, et al. Minocycline extended-release comparison with doxycycline for the treatment of rosacea: a randomized, head-to-head, clinical trial. *J Clin Aesthet Dermatol.* 2021;14(12):16-23.
16. Sanchez ML, Jones RN. E test, an antimicrobial susceptibility testing method with broad clinical and epidemiologic application. *Antimicrobic Newsletter.* 1992;8(1):1-7.
17. Del Rosso JQ, Thiboutot D, Gallo R, et al. Consensus recommendations from the American Acne & Rosacea Society on the management of rosacea, part 3: a status report on systemic therapies. *Cutis.* 2014;93(1):18-28.
18. Schaller M, Almeida LMC, Bewley A, et al. Recommendations for rosacea diagnosis, classification and management: update from the global ROSacea COnsensus 2019 panel. *Br J Dermatol.* 2020;182(5):1269-1276.
19. Dethlefsen L, Relman DA. Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation. *Proc Natl Acad Sci U S A.* 2011;108 Suppl 1(Suppl 1):4554-4561.
20. Elvers KT, Wilson VJ, Hammond A, et al. Antibiotic-induced changes in the human gut microbiota for the most commonly prescribed antibiotics in primary care in the UK: a systematic review. *BMJ Open.* 2020;10(9):e035677.
21. Oracea (doxycyline) prescribing information. In. Dallas, TX: Galderma Laboratories, LP; 2022.
22. Del Rosso JQ. Clinical significance of brand versus generic formulations: focus on oral minocycline. *Cutis.* 2006;77(3):153-156.
23. Fowler JF. Anti-inflammatory dose doxycycline for the treatment of rosacea. *Expert Review of Dermatology.* 2007;2(5):523-531.
24. Preshaw PM, Novak MJ, Mellonig J, et al. Modified-release subantimicrobial dose doxycycline enhances scaling and root planing in subjects with periodontal disease. *J Periodontol.* 2008;79(3):440-452.
25. Chien AL, Tsai J, Leung S, et al. Association of Systemic Antibiotic Treatment of Acne With Skin Microbiota Characteristics. *JAMA Dermatol.* 2019;155(4):425-434.
26. Thompson KG, Rainer BM, Antonescu C, et al. Minocycline and its impact on microbial dysbiosis in the skin and gastrointestinal tract of acne patients. *Ann Dermatol.* 2020;32(1):21-30.

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