POSTER NUMBER

2106

Changes in Antimicrobial Susceptibility of Culturable Fecal Bacteria During Rifaximin Treatment for the Prevention of Overt Hepatic Encephalopathy (HE) Recurrence

Herbert L. DuPont, MD[1]; Steven L. Flamm, MD[2]; Ray A. Wolf, PharmD[3]; Arun J. Sanyal, MD[4]

[1]University of Texas School of Public Health and Medical School, Kelsey Research Foundation, and Baylor College of Medicine, Houston, TX; [2]Northwestern University Feinberg School of Medicine, Chicago, IL; [3]Salix Pharmaceuticals, Raleigh, NC; [4]Virginia Commonwealth University and McGuire VA Medical Center, Richmond, VA

INTRODUCTION

- Rifaximin is an oral nonsystemic, gastrointestinal-targeted antibiotic indicated for reducing the risk of overt HE recurrence in adults; in a randomized, double-blind, placebo-controlled clinical trial,[1] rifaximin 550 mg twice daily (BID) demonstrated a:
  - 58% reduction in the relative risk of experiencing overt HE recurrence (hazard ratio [HR], 0.42; 95% confidence interval [CI], 0.28–0.64; $P<0.001$)
  - 50% reduction in the relative risk of an HE-related hospitalization during a 6-month period versus placebo (HR, 0.50; 95% CI, 0.29–0.87; $P=0.01$)
- The risk of bacterial antibiotic resistance to rifaximin is thought to be low, possibly because of the minimal systemic absorption of rifaximin,[2,3] the requirement of a stable mutation in host cell DNA (in contrast with plasmid-based mechanisms),[4] and the inability of resistant bacteria to effectively colonize the gastrointestinal tract[5,6]
- However, stool microbiota characterization data are limited in a population of patients with cirrhosis receiving daily rifaximin therapy

AIM

- To assess the impact of a 6-month daily course of rifaximin therapy on the antibiotic susceptibility profile of stool bacteria in patients with cirrhosis and history of HE

METHODS

- Randomized, phase 4, open-label, active-controlled trial conducted in adults with cirrhosis, with history of ≥1 episode of overt HE within 6 months prior to screening, and were currently in HE remission (Conn score 0 or 1)
  - No active spontaneous bacterial peritonitis infection or other current infection for which the patient was taking oral or parenteral antibiotics and no positive stool test for *Clostridium difficile* (toxin A or B) at screening
- Patients were randomly assigned to receive open-label rifaximin 550 mg BID alone or rifaximin 550 mg BID + lactulose (self-titrated, 2–3 soft stools/day) for 6 months
- All patients provided stool samples at baseline and at end of treatment (EOT), and randomly selected samples from select sites were included in the current analysis
- Bacteria were cultured and minimum inhibitory concentrations (MIC) were determined using standard techniques; previously defined breakpoints were used to determine resistance for antibiotics tested[7]
- Susceptibility testing was performed for 11 antibiotics, including rifaximin and rifampin
- P values for change from baseline in bacterial fractions were determined using 1-sample (within treatment) or 2-sample (between treatment) Wilcoxon tests on the log (postbaseline bacterial fractions/baseline bacterial fractions) and corrected for multiple hypothesis testing with Benjamini-Hochberg method

RESULTS

- 64 patients were included in the current stool microbiota analysis (Table 1)

Table 1. Demographics and Baseline Characteristics

Characteristic	Rifaximin 550 mg BID (n=31)	Rifaximin 550 mg BID + lactulose (n=33)
Age, y, mean (SD)	56.2 (8.8)	55.4 (9.4)
Range	36-71	35-70
Male, %	61.3	66.7
Race, n (%)		
White	30 (96.8)	30 (90.9)
Black	0	2 (6.1)
Other/unknown	1 (3.2)	1 (3.0)
Child-Pugh classification, n (%)		
Class A	16 (51.6)	11 (33.3)
Class B	15 (48.4)	21 (63.6)
Class C	0	1 (3.0)
MELD score, mean (SD)	10.6 (3.0)	11.8 (2.8)

BID = twice daily; MELD = Model for End-Stage Liver Disease; SD = standard deviation.

- Overall, patients in both treatment groups had a similar distribution of bacterial families and species at baseline and at EOT
  - Bacteria in the Enterobacteriaceae, Enterococcaceae, and Bacteroidaceae families were the most commonly isolated types in both groups (Table 2)
  - The most frequently identified bacterial species across treatment groups and timepoints was *Escherichia coli* (93 isolates; 24.7%); all other bacterial species had an overall frequency of <8.5%
  - For *C difficile* and *Enterococcus faecalis*, there were fewer isolates recovered post-treatment with rifaximin in both groups
  - The number of *Enterococcus faecium* isolates was generally similar at baseline (pre-) and post-treatment in both groups

Table 2. Overall Collection of Stool Bacterial Isolates Obtained During Study[a]

	Rifaximin 550 mg BID		Rifaximin 550 mg BID + lactulose	
Isolates, n (%)	Baseline (n=103)	EOT (n=80)	Baseline (n=92)	EOT (n=101)
Bacteroidaceae	21 (20.4)	23 (28.8)	22 (23.9)	30 (29.7)
Bacteroides fragilis	3 (2.9)	4 (5.0)	5 (5.4)	9 (8.9)
Bacteroides thetaiotaomicron	4 (3.9)	8 (10.0)	3 (3.3)	3 (3.0)
Bacteroides uniformis	4 (3.9)	2 (2.5)	6 (6.5)	7 (6.9)
Bacteroides vulgatus	5 (4.9)	4 (5.0)	4 (4.3)	5 (5.0)
Parabacteroides distasonis	3 (2.9)	4 (5.0)	3 (3.3)	4 (4.0)
Other	2 (1.9)	1 (1.3)	1 (1.1)	2 (2.0)
Clostridiaceae[b]				
Clostridium difficile	7 (6.8)	1 (1.3)	4 (4.3)	1 (1.0)
Enterobacteriaceae	35 (34.0)	32 (40.0)	33 (35.9)	36 (35.6)
Escherichia coli	27 (26.2)	19 (23.8)	23 (25.0)	24 (23.8)
Klebsiella oxytoca	2 (1.9)	5 (6.3)	2 (2.2)	1 (1.0)
Klebsiella pneumoniae	5 (4.9)	6 (7.5)	8 (8.7)	11 (10.9)
Other	1 (1.0)	2 (2.5)	0 (0)	0 (0)
Enterococcaceae	30 (29.1)	18 (22.5)	29 (31.5)	27 (26.7)
Enterococcus avium	6 (5.8)	3 (3.8)	9 (9.8)	7 (6.9)
Enterococcus casseliflavus	3 (2.9)	3 (3.8)	3 (3.3)	2 (2.0)
Enterococcus durans	2 (1.9)	1 (1.3)	2 (2.2)	3 (3.0)
Enterococcus faecalis	10 (9.7)	1 (1.3)	7 (7.6)	4 (4.0)
Enterococcus faecium	7 (6.8)	9 (11.3)	8 (8.7)	7 (6.9)
Other	2 (1.9)	1 (1.3)	0 (0)	4 (4.0)
Staphylococcaceae	10 (9.7)	6 (7.5)	4 (4.3)	7 (6.9)
Staphylococcus aureus	5 (4.9)	0 (0)	3 (3.3)	2 (2.0)
Staphylococcus epidermidis	5 (4.9)	5 (6.3)	0 (0)	3 (3.0)
Other	0 (0)	1 (1.3)	1 (1.1)	2 (2.0)

[a]Collected at baseline and end of treatment.
[b]*C difficile* was the only *Clostridium* species cultured.
BID = twice daily; EOT = end of treatment.

- Overall, there were no significant differences in mean change from baseline to end of treatment in bacterial fractions within or between the 2 treatment groups for taxa present in ≥25% of population analyzed (data not shown; $P>0.05$)
  - The only significant difference was with change from baseline to EOT for Enterococcaceae fraction in the rifaximin + lactulose group ($P=0.02$ vs baseline)
- In general, antibiotic susceptibility did not differ substantially from baseline to EOT in both groups for the most common bacterial families (Table 3)
  - MIC_{50} values for rifampin and rifaximin, but not the other antibiotics tested, were generally higher at EOT
- Cross-resistance to other antibiotics rarely developed (Table 4)
  - The one *C difficile* isolate obtained post-treatment in the rifaximin-alone group acquired resistance to rifaximin, but not vancomycin or fidaxomicin, the 2 main antibiotics used to treat *C difficile* infection

Table 3. Antibiotic Susceptibility of Select Stool Bacterial Isolates

	Rifaximin 550 mg BID				Rifaximin 550 mg BID + lactulose			
	MIC_{50} (μg/mL)		MIC_{90} (μg/mL)		MIC_{50} (μg/mL)		MIC_{90} (μg/mL)	
Family and antibiotic tested	Baseline	EOT	Baseline	EOT	Baseline	EOT	Baseline	EOT
Bacteroidaceae (# isolates)	(n=21)	(n=23)	(n=21)	(n=23)	(n=22)	(n=30)	(n=22)	(n=30)
Fidaxomicin	>128	>128	>128	>128	>128	>128	>128	>128
Metronidazole	1	1	2	2	1	1	2	2
Rifampin	0.25	>64	>64	>64	>64	>64	>64	>64
Rifaximin	0.5	1024	1024	>1024	256	1024	>1024	>1024
Vancomycin	64	64	128	128	64	32	128	128
Clostridiaceae (# isolates)[a]	(n=7)		(n=7)		(n=4)		(n=4)	
Fidaxomicin	0.25		>128		0.25		0.5	
Metronidazole	0.5	–	8	–	0.5	–	0.5	–
Rifampin	≤0.015		0.06		≤0.015		≤0.015	
Rifaximin	0.03		0.12		0.03		0.03	
Vancomycin	0.25		32		0.25		0.5	
Enterobacteriaceae (# isolates)	(n=35)	(n=32)	(n=35)	(n=32)	(n=33)	(n=36)	(n=33)	(n=36)
Ceftazidime	0.12	0.12	0.25	0.5	0.12	0.12	0.5	1
Ceftriaxone	≤0.03	≤0.03	0.06	0.12	≤0.03	≤0.03	0.06	0.5
Ciprofloxacin	0.015	0.015	>8	2	0.015	0.015	0.25	>8
Imipenem	0.12	0.12	0.12	0.25	0.12	0.06	0.12	0.12
Meropenem	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015
TZP[b]	1	2	4	4	2	2	8	8
Rifampin	16	128	>128	>128	32	>128	>128	>128
Rifaximin	16	>128	>128	>128	32	>128	>128	>128
Enterococcaceae (# isolates)	(n=30)	(n=18)	(n=30)	(n=18)	(n=29)	(n=27)	(n=29)	(n=27)
Ceftazidime	>64	>64	>64	>64	>64	>64	>64	>64
Ceftriaxone	>64	32	>64	>64	32	>64	>64	>64
Ciprofloxacin	0.5	1	2	>8	0.5	0.5	2	>8
Imipenem	0.5	0.5	4	>32	0.5	1	4	>32
Meropenem	4	4	8	>8	4	8	>8	>8
TZP[b]	8	8	32	>64	8	16	32	>64
Rifampin	0.5	64	64	>128	2	>128	>128	>128
Rifaximin	1	>128	>128	>128	2	>128	>128	>128
Staphylococcaceae (# isolates)	(n=10)	(n=6)	(n=10)	(n=6)	(n=4)	(n=7)	(n=4)	(n=7)
Ceftazidime	8	8	16	16	16	8	16	16
Ceftriaxone	2	1	2	8	2	2	16	16
Ciprofloxacin	0.12	0.12	8	>8	0.25	0.12	8	>8
Imipenem	≤0.015	≤0.015	0.03	0.06	≤0.015	≤0.015	0.03	0.03
Meropenem	0.06	0.06	0.06	0.5	0.06	0.06	0.25	2
TZP[b]	0.5	0.25	1	1	1	0.5	16	1
Rifampin	≤0.06	>128	≤0.06	8	1	>128	≤0.06	128
Rifaximin	≤0.06	>128	1	>128	≤0.06	8	≤0.06	>128

[a]Clostridiaceae only had 1 isolate identified at EOT in each group and MIC_{50} and MIC_{90} were not determined. [b]For TZP, MIC values reported are for piperacillin; tazobactam concentrations remained constant at 4 μg/mL at all dilutions. BID = twice daily; EOT = end of treatment; MIC = minimum inhibitory concentration; MIC_{50} = MIC required to inhibit the growth of 50% of microorganisms; MIC_{90} = MIC required to inhibit the growth of 90% of microorganisms; TZP = piperacillin/tazobactam.

Table 4. Susceptibility Profile of Bacteria to Various Antibiotics

	Resistant Isolates, n			
	Rifaximin 550 mg BID		Rifaximin 550 mg BID + lactulose	
Antibiotic tested[a]	Baseline	EOT	Baseline	EOT
Bacteroidaceae	(n=21)	(n=23)	(n=22)	(n=30)
Fidaxomicin	21	23	22	30
Metronidazole	0	0	0	0
Rifampin	7	23	12	30
Rifaximin	6	14	9	20
Vancomycin	7	4	9	11
Clostridiaceae	(n=7)	(n=1)	(n=4)	(n=1)
Fidaxomicin	1	0	0	0
Metronidazole	0	0	0	0
Rifampin	0	1	0	0
Rifaximin	0	1	0	0
Vancomycin	0	0	0	0
Enterobacteriaceae	(n=35)	(n=32)	(n=33)	(n=36)
Ceftazidime	1	1	0	2
Ceftriaxone	1	1	1	3
Ciprofloxacin	4	3	3	4
Imipenem	0	0	0	0
Meropenem	0	0	0	0
TZP	0	0	0	0
Rifampin	8	18	11	22
Rifaximin	10	25	14	26
Enterococcaceae	(n=30)	(n=18)	(n=29)	(n=27)
Ceftazidime	26	16	23	23
Ceftriaxone	16	7	14	15
Ciprofloxacin	3	6	2	4
Imipenem	2	3	0	4
Meropenem	10	8	12	14
TZP	2	3	2	6
Rifampin	10	18	13	26
Rifaximin	5	11	6	18
Staphylococcaceae	(n=10)	(n=6)	(n=4)	(n=7)
Ceftazidime	0	0	0	0
Ceftriaxone	0	0	0	0
Ciprofloxacin	2	3	2	1
Imipenem	0	0	0	0
Meropenem	0	0	0	0
TZP	0	0	1	0
Rifampin	1	6	0	4
Rifaximin	0	5	0	2

[a]All MIC values less than the assigned breakpoint were considered susceptible. The assigned breakpoint was either the CLSI established breakpoint, or for antibiotics without a CLSI established breakpoint, the highest dilution that was tested.
BID = twice daily; CLSI = Clinical Laboratory Standards Institute; EOT = end of treatment; MIC = minimum inhibitory concentration; TZP = piperacillin/tazobactam.

CONCLUSION

- **Rifaximin alone or rifaximin plus lactulose administered daily for up to 6 months did not substantially alter the susceptibility of stool bacteria to non-rifamycin antibiotics**

REFERENCES: **1.** Bass NM, et al. *N Engl J Med.* 2010;362(12):1071-1081. **2.** Taylor DN, et al. *Antimicrob Agents Chemother.* 2008;52(9):1179-1181. **3.** Descombe JJ, et al. *Int J Clin Pharmacol Res.* 1994;14(2):51-56. **4.** Kothary V, et al. *Antimicrob Agents Chemother.* 2013;57(2):811-817. **5.** De Leo C, et al. *Drugs Exp Clin Res.* 1986;12(12):979-981. **6.** Brigidi P, et al. *J Chemother.* 2002;14(3):290-295. **7.** Clinical Laboratory Standards Institute document M100-S24. 2014.

ACKNOWLEDGMENTS: Technical editorial and medical writing assistance was provided under the direction of the authors by Mary Beth Moncrief, PhD, Synchrony Medical Communications, LLC, West Chester, PA. Funding for this support was provided by Salix Pharmaceuticals, Raleigh, NC.

DISCLOSURES: HLD, SLF, and AJS have served as a consultant or advisor for Salix Pharmaceuticals. RAW is a former employee of Salix Pharmaceuticals.

The Liver Meeting® 2016, American Association for the Study of Liver Diseases • November 11–15, 2016 • Boston, MA

Research funded by: Salix Pharmaceuticals