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Activity of DS-2969b, a novel GyrB inhibitor, against recent clinical isolates of *Clostridium difficile* from Europe

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Abstract

Objectives: DS-2969b (DS) is a novel GyrB inhibitor currently in early development for the treatment of *C. difficile* infections. This study was undertaken to determine DS activity against recent clinical isolates of *C. difficile* from Europe collected in 2015.

Methods: Isolates (n=108) originally collected from European laboratories in 2015 were selected randomly from the IHMA repository. These originated from ten countries (Belgium [n=4], Czech Republic [n=4], France [n=24], Germany [n=21], Hungary [n=3], Poland [n=4], Romania [n=15], Spain [n=19], Sweden [n=5] and the United Kingdom [n=9]). The minimum inhibitory concentration (MIC) for DS and comparators (vancomycin [VAN], metronidazole [MTZ], fidaxomicin [FDX] and moxifloxacin [MFX]) was determined by CLSI anaerobic agar dilution methodology (CLSI M11-A8) and susceptibility (MFX & MTZ only) was determined according to CLSI breakpoints (CLSI M100-S26). PCR-ribotyping was performed at the *C. difficile* Ribotyping Network for England and Northern Ireland (CDRN) Reference Laboratory, Leeds, UK.

Results: A narrow DS MIC range of between 0.03 and 0.12 µg/mL was seen against 108 *C. difficile* clinical isolates from Europe. The most prevalent ribotype was 027 (21% of isolates) followed by 078 (9%). Summary MIC data are shown in the Table below. DS MIC was unaffected by resistance to MFX, but MIC was slightly increased against ribotype 027 isolates (MIC50 increased from 0.06 µg/ml to 0.12 µg/ml) - an effect shared by MTZ, FDX and MFX (but not VAN). No MTZ-resistant isolates were observed.

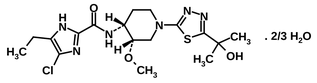
		MIC (µg/ml):				
		DS	VAN	MTZ	FDX	MFX
ALL (n=108)	MIC_{50}	0.06	1	0.25	0.25	2
	MIC_{90}	0.12	2	1	1	>16
027 ribotype (n=23)	MIC_{50}	0.12	1	1	0.5	>16
	MIC_{90}	0.12	2	1	1	>16
MFX-Resistant (n=50)	MIC_{50}	0.06	1	1	0.5	>16
	MIC_{90}	0.12	2	1	1	>16

Conclusions: DS showed excellent activity against the clinical isolates tested with lower MICs than the comparators: FDX, MTZ, VAN and MFX. This study shows potential for DS-2969b as a new *C. difficile* agent and warrants further investigation.

Introduction

DS-2969b, a novel GyrB inhibitor, from Daiichi Sankyo Co. Ltd. (Japan), has narrow-spectrum antimicrobial activity against Gram-positive bacteria, including strict anaerobes and facultative anaerobes. DS-2969b is currently in early development for the treatment of *C. difficile* infections.

Fig.1 Chemical structure of DS-2969b



Materials & Methods

- A total of 108 bacterial isolates collected in 2015 from 10 European countries were evaluated (Table 1).
- All isolates were ribotyped at the CDRN Reference Laboratory, Leeds, UK by PCR amplification of the 16S-23S intergenic spacer regions and size of fragments determined, essentially as described previously (1), with modifications.
- PCR-ribotypes were assigned by comparison with those in the CDRN UK PCR-ribotype library
- Minimum inhibitory concentrations (MICs) for DS-2969b and antimicrobial comparators were determined by agar dilution following Clinical and Laboratory Standards Institute (CLSI) guidelines (2).
- MIC_{50} and MIC_{90} (concentrations to inhibit 50% & 90% of isolates, respectively) were calculated.
- CLSI breakpoints (3) were used to determine the susceptibility of isolates to metronidazole and moxifloxacin. Metronidazole breakpoints (µg/ml) are ≤8 (Sus), 16 (Int) and ≥32 (Res). Moxifloxacin CLSI breakpoints (µg/ml) are ≤2 (Sus), 4 (Int) and ≥8 (Res). Susceptibility to other agents was not determined due to a lack of CLSI breakpoints.

Table 1. Clinical isolates of *C. difficile* included in the study

Country	# isolates per country
Belgium	4
Czech Republic	4
France	24
Germany	21
Hungary	3
Poland	4
Romania	15
Spain	19
Sweden	5
United Kingdom	9
Grand Total	108

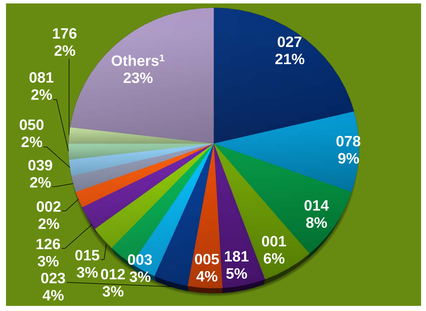
References

1. Fawley, W.N. *et al.* 2015. Development and validation of an internationally-standardized, high resolution capillary gel-based electrophoresis PCR-ribotyping protocol for *Clostridium difficile*. PLoS ONE 10(2): e0118150.
2. CLSI. 2012. Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria; Approved Standard-Eighth Edition. CLSI document M11-A8.
3. CLSI. 2016. Performance Standards for Antimicrobial Susceptibility Testing. Twenty-sixth Edition. CLSI Supplement M100S

Results

- The isolates tested included a wide range of ribotypes, as shown in Fig. 2.
- Ribotype 027 was the most commonly observed (23/108 isolates, 21%).
- DS-2969b showed a narrow MIC distribution between 0.03 and 0.12 µg/ml and had the lowest MIC of all the antimicrobial agents tested (Table 2).
- A high percentage of isolates (46.3%) were resistant to moxifloxacin (Table 2) but the activity of DS-2969b was unaffected by this resistance (Table 3).
- DS-2969b was also very active against ribotype 027 isolates although MIC_{50} was slightly increased, as also observed with metronidazole and fidaxomicin (Table 4), compared to the total *C. difficile* population (Table 2).
- Almost all 027 ribotype isolates were resistant to moxifloxacin (Table 4)

Fig.2 Ribotype distribution for 108 isolates from European countries



[1]Others include one isolate each of ribotype 009, 010, 011, 019, 020, 026, 029, 045, 046, 056, 062, 070, 071, 087, 090, 097, 193, 198, 216, 220, 344, 644, 774, 789 & 799

Conclusions

- DS-2969b showed excellent activity against the clinical isolates tested, including ribotype 027 isolates and moxifloxacin-resistant isolates, with lower MICs than fidaxomicin, metronidazole, vancomycin or moxifloxacin.
- This study shows potential for DS-2969b as a new *C. difficile* agent and warrants further investigation.

Acknowledgments

- This study was sponsored by Daiichi Sankyo India Pharma Pvt. Ltd.
- We thank CDRN for performing the isolate ribotyping.

Table 2. Summary MIC and susceptibility data for DS-2969b and comparators against 108 clinical isolates of *C. difficile*

Antimicrobial Agent	MIC (µg/mL):				Susceptibility		
	MIC_{50}	MIC_{90}	Min	Max	% Sus	% Int	% Res
DS-2969b	0.06	0.12	0.03	0.12	-	-	-
Vancomycin	1	2	0.5	4	-	-	-
Metronidazole[1]	0.25	1	0.25	2	100.0	0.0	0.0
Fidaxomicin	0.25	1	0.03	1	-	-	-
Moxifloxacin[2]	2	>16	1	>16	53.7	0.0	46.3

Table 3. Summary MIC and susceptibility data for DS-2969b and comparators against 50 moxifloxacin-resistant clinical isolates of *C. difficile*

Antimicrobial Agent	MIC (µg/mL):				Susceptibility		
	MIC_{50}	MIC_{90}	Min	Max	% Sus	% Int	% Res
DS-2969b	0.06	0.12	0.03	0.12	-	-	-
Vancomycin	1	2	0.5	4	-	-	-
Metronidazole[1]	1	1	0.25	2	100.0	0	0
Fidaxomicin	0.5	1	0.03	1	-	-	-
Moxifloxacin[2]	>16	>16	8	>16	0.0	0.0	100.0

Table 4. Summary MIC and susceptibility data for DS-2969b and comparators against 23 ribotype 027 clinical isolates of *C. difficile*

Antimicrobial Agent	MIC (µg/mL):				Susceptibility		
	MIC_{50}	MIC_{90}	Min	Max	% Sus	% Int	% Res
DS-2969b	0.12	0.12	0.06	0.12	-	-	-
Vancomycin	1	2	1	4	-	-	-
Metronidazole[1]	1	1	0.25	1	100.0	0	0
Fidaxomicin	0.5	1	0.25	1	-	-	-
Moxifloxacin[2]	>16	>16	2	>16	4.3	0.0	95.7