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Freeman, J, Pilling, S, Vernon, J et al. (1 more author) (2017) In vitro activities of MCB3681 and 8 comparators against Clostridium difficile isolates with known ribotypes and diverse geographical spread. Antimicrobial Agents and Chemotherapy, 61 (3). e02077-16. ISSN 0066-4804

https://doi.org/10.1128/AAC.02077-16

In vitro activities of MCB3681 and 8 comparators against Clostridium difficile isolates with known ribotypes and diverse geographical spread

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Running title: C. difficile susceptibility to MCB3681 and comparators

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Abstract

Treatments for Clostridium difficile infection remain limited, despite the introduction of fidaxomicin, and development of new agents is necessary.

We determined the in vitro susceptibilities of 199 prevalent or emerging Clostridium difficile PCR ribotypes to MCB3681, a novel investigational quinolonyl-oxazolidinone, and 8 comparators (metronidazole, vancomycin, fidaxomicin, moxifloxacin, ciprofloxacin, clindamycin, tigecycline and linezolid).

MCB3681 showed good activity against C. difficile with no evidence of MCB3681 resistance in isolates showing either or both moxifloxacin and linezolid resistance.

C. difficile infection (CDI) is a major burden on healthcare resources. CDI is thought to arise following the depletion of gut microflora by antimicrobial action, allowing the organism to proliferate and cause disease. Antimicrobial treatments for CDI are currently limited to metronidazole, vancomycin and fidaxomicin. Metronidazole has more recently been associated with treatment failures, while promotion of glycopeptide resistance within the host microflora is a risk associated with vancomycin therapy. [1] Symptomatic recurrence is common following treatment with these agents, [2] requiring further episodes of antimicrobial therapy. Further treatment options are highly desirable to broaden the range of therapeutic choice and strengthen antimicrobial stewardship.

MCB3681 is a novel small molecule with structural elements of an oxazolidinone and a quinolone showing good activity against C. difficile, including isolates that were resistant to linezolid, ciprofloxacin, moxifloxacin and clindamycin. [3] It achieves high faecal concentrations after intravenous infusions and has shown activity against Gram positive components of the gut microflora in a clinical Phase 1 study [4]. The development of an intravenous treatment agent achieving high faecal concentrations would circumvent issues of rapid gut transit, or impaired delivery of orally administered agents due to ileus, particularly in patients with severe or protracted/multiple recurrent diarrhoeal episodes.

We determined the in vitro activities of MCB3681 and 8 comparators (metronidazole, vancomycin, moxifloxacin, ciprofloxacin, clindamycin, tigecycline, linezolid, and fidaxomicin) against a panel of 200 Clostridium difficile isolates of known PCR ribotypes (RT) from 21 European countries (selected from the ClosER study - July 2011-April 2013, by kind permission of Astellas Pharma Europe). [5]

In vitro susceptibility testing was performed using a Wilkins-Chalgren agar incorporation method, as previously described.[5,7] Briefly, C. difficile test isolates and control strains (C. difficile ATCC 750057, C. difficile E4 PCR ribotype 010, Bacteroides fragilis ATCC 25285, Enterococcus faecalis ATCC 29212 and Staphlyococcus aureus ATCC 29213 were cultured anaerobically at 37^{o}C for 24h in Schaedler's anaerobic broths prior to dilution to 0.5 McFarland standard equivalence) in pre-reduced sterile saline and inoculation onto antibiotic-containing and control Wilkins-Chalgren agar plates. Inoculated plates were incubated anaerobically at 37^{o}C for 48h.

MCB3681 is a quinolonyl- oxazolidinone antibacterial which has previously demonstrated good activity against C. difficile. [3] All the CDI treatment agents, including MCB3681 showed good activity against the isolates tested (Table 1). Fidaxomicin was the most active treatment agent (Kruskal-Wallis $p<0.0001$; geometric mean (GM) MIC=0.05 mg/L), followed by MCB3681 ($p<0.0001$; GM=0.12 mg/L), then metronidazole ($p<0.0001$; GM=0.33 mg/L), with no evidence of resistance to any of these compounds (Table 1). Vancomycin was the least active ($p<0.0001$; GM=1.02 mg/L), but resistance was very scarce (1.5%; breakpoint>8mg/L). Reduced metronidazole susceptibility (4 mg/L) was observed in only 1% of isolates. GM metronidazole MICs were elevated in RT027 (0.96 mg/L) and RT106 (0.74 mg/L) vs GM metronidazole MICs for all isolates tested (0.33 mg/L), in agreement with previous data.[4]

All isolates were resistant to ciprofloxacin according to the breakpoints defined (Table 1), and 48% of isolates showed moxifloxacin resistance, including at least one isolate in each RT group tested. Highly elevated MICs to both moxifloxacin ($\geq$32 mg/L) and ciprofloxacin ($\geq$128 mg/L) were prevalent in RT001, RT027 and RT356. Clindamycin MICs were highest in RT001, RT017 and RT126 (GM MICs = 61.11 mg/L; 64 mg/L and 38.05 mg/L, respectively), but there was evidence of clindamycin resistance in all RTs tested (Table 1). There was no evidence of tigecycline resistance (range=0.03-0.125mg/L; GM=0.05mg/L), in agreement with previous data (Table 1).[4] The majority of isolates (78.9%) were sensitive to linezolid (Table 1), with a GM MIC of 5.16 mg/L. RT001 and RT017 showed the highest GM linezolid MICs (10.08 mg/L and 7.03 mg/L, respectively). This is also in agreement with previous observations.[7] Three RT017, and two RT027 isolates showed dual quinolone-oxzolidinone resistant phenotype, and showed MCB3681 MICs of 0.5 mg/L. We

have previously reported that these isolates showed high level resistance to chloramphenicol (Table 2).[5,8] Marin et al. reported linezolid, chloramphenicol, erythromycin, and clindamycin resistance associated with the presence of the multidrug resistance gene, cfr, in C. difficile RT017, RT078 and RT126 isolates.[11] The MIC_{50} and MIC_{90} values reported here for MCB3681 are similar to those recently described for cadazolid, another quinolonyl-oxazolidinone molecule.[9] A previous study investigating susceptibility of C. difficile to cadazolid and comparators, reported an association between resistance to either moxifloxacin or linezolid and moxifloxacin/linezolid double-resistant mutants, and 2- or 4-fold higher cadazolid MICs in mono- or double-resistant isolates, respectively. [10] However, the highest MCB3681 MIC was 0.5 mg/L, and we also found isolates with moxifloxacin, ciprofloxacin, linezolid and chloramphenicol resistance that demonstrated very low MCB3681 MICs (0.008 mg/L) (Table 2). We did not investigate the molecular basis of resistance in these isolates, but the results do not suggest a link between this phenotype and MCB3681 MICs. The results shown here, in conjunction with those previously reported[8, 11] would also indicate that other modes of resistance to linezolid (23s rRNA alterations, ribosomal protein modifications) may be at play in combination with quinolone resistance mechanisms.

Rashid et al. reported MICs of MCB3681 for C. difficile ranged from 0.008-0.5 mg/L,[3] which were similar to our results (range 0.008-0.5 mg/L). However, in the present study, MIC_{50} and MIC_{90} values were 0.125 and 0.25 mg/L, respectively, which were marginally higher than those reported previously, but within 2 doubling dilutions (0.03 and 0.06 mg/L, respectively). This may be explained by methodological/agar or C. difficile strain distribution differences. The influence of testing media and components therein on MICs has previously been reported and may have been a factor in the differences observed.[7,12] We used a Wilkins-Chalgren agar incorporation method to determine MICs, since is superior to CLSI-recommended Brucella blood agar (BBA) in the detection of reduced susceptibility to metronidazole in C. difficile.[7]

This study builds on this previous data of Rashid et al by substantially expanding the diversity of ribotypes examined to include, in particular, RT027 and several RTs already noted for resistance to multiple antimicrobials: RT001, RT017, RT018, RT027 and RT356.,[5,8] There was no evidence of MCB3681 resistance among them. MCB3681 achieves faecal concentrations of 99-226mg/kg after intravenous

infusions, far in excess of MIC ranges for C. difficile reported here. MCB3681 has been reported to be active against Gram-positive gut microflora bacteria, but sparing of Gram-negative organisms in human volunteer studies with intravenous administration over 5 days. Further data are needed to assess the impact of MCB3681 on C. difficile and the gut microflora over a longer duration.

In summary, MCB3681 showed good activity against C. difficile isolates from emerging or prevalent European PCR ribotypes with no evidence of resistance. The presence of quinolone and/or linezolid resistance did not influence MCB3681 MICs.

Funding

This research was funded by Morphochem AG, Munich, Germany

Acknowledgements

We are grateful to Dr Chris Longshaw and Astellas Pharma Europe for kind permission to use C. difficile isolates collected during The ClosER study.

Transparency Declaration

JF has grant/research funding outside this work from Astellas and Melinta Therapeutics.

MHW has received grant/research funding outside this work from Abbott, Actelion, Alere, Astellas, Biomerieux, Cerexa, Cubist, Da Volterra, European Tissue Symposium, Merck, Sanofi-Pasteur, Summit, The Medicines Company and Qiagen and consultancies and/or lecture honoraria from Actelion, Alere, Astellas, Astra-Zeneca, Basilea, Bayer, Cubist, Durata, European Tissue Symposium, J&J, Merck, Nabriva, Novacta, Novartis, Optimer, Pfizer, Roche, Sanofi-Pasteur and Seres and has been a member of a speaker's bureau for Pfizer.

JV and SP have nothing to declare.

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mg/L	MCB3681	FDX	MTZ	VAN	MXF	CIP	CLI	TGC	LZD
Breakpoints	S<4; R>4[2]	S <1; RS >1[4]	S<2; I=4; R>8[4]	S<2; I=4; R>8[4]	S<2; I=4; R>8[4]	S <8; RS >8[2]	S<2; I=4; R>8[4]	S<4; RS>4[4]	S<4; R>4[7]
%S	100	100	99	96	50.5	-	5.5	100	78.9
%I	-	-	1	2.5	1	-	29.5	-	-
%R	-	-	0	1.5	48	100	54	-	21.1
MIC_{50}	0.125	0.06	0.25	1	2	64	16	0.06	4
MIC_{90}	0.25	0.125	1	2	32	256	128	0.06	8
range	0.008-0.5	0.004-0.25	<0.125-4	0.5-8	1->64	8->128	1->64	0.03-0.125	2->64
Geometric mean MIC (mg/L)									
RT001 (15)	0.07	0.02	0.42	0.79	16.00	111.43	61.11	0.03	10.08
RT002 (14)	0.11	0.06	0.19	0.87	1.82	27.86	12.13	0.04	4.39
RT005 (16)	0.14	0.06	0.29	1.16	2.00	37.12	9.28	0.04	5.66
RT014 (16)	0.11	0.07	0.28	0.88	3.36	39.74	10.37	0.05	4.36
RT015 (15)	0.14	0.06	0.25	0.87	1.91	26.60	7.29	0.04	4.19
RT017 (16)	0.15	0.04	0.26	0.74	12.88	86.67	64.00	0.06	7.03
RT018 (14)	0.12	0.06	0.41	1.49	6.90	110.33	8.83	0.04	4.42
RT020 (15)	0.10	0.06	0.25	0.75	2.59	36.44	11.31	0.05	4.76
RT027 (16)	0.16	0.09	0.96	1.14	21.67	206.14	19.87	0.05	5.19
RT078 (16)	0.11	0.05	0.26	0.92	2.38	34.90	12.34	0.05	5.42
RT106 (14)	0.11	0.09	0.74	1.10	7.61	81.98	10.77	0.04	4.42
RT126 (16)	0.12	0.06	0.32	1.00	8.35	72.88	38.05	0.06	4.56
RT356 (16)	0.08	0.04	0.27	2.28	29.34	245.15	12.88	0.04	4.76
All isolates (199)	0.12	0.05	0.33	1.02	5.87	66.27	16.17	0.05	5.16

Table 1. Susceptibility of 199 C. difficile isolates to MCB3681 and 8 comparators.
FDX= fidaxomicin; MTZ=metronidazole, VAN= vancomcyin; MXF=moxifloxacin;CIP=ciprofloxacin; CLI=clindamycin; TGC=tigecycline; LZD=linezolid
S=sensitive; I=intermediate; R=resistant; RS=reduced susceptibility

	MIC (mg/L)					
RT	MXF	CIP	LZD	CLI	CHL	MCB3681
001	16	128	32	>64	32	0.008
001	32	128	32	>64	32	0.015
001	32	128	32	>64	32	0.015
001	16	64	16	>64	8	0.25
001	16	>128	32	>64	8	0.25
001	16	>128	32	>64	32	0.25
001	16	>128	32	>64	32	0.25
014	16	>128	32	16	16	0.06
017	32	128	64	>64	2	0.06
017	16	64	16	>64	32	0.25
017	32	64	16	>64	64	0.5
017	32	64	32	>64	>64	0.5
017	32	128	32	>64	64	0.5
018	16	>128	8	8	4	0.03
018	32	>128	8	8	2	0.06
027	32	>128	32	>64	64	0.5
027	32	>128	32	>64	64	0.5
078	8	128	8	4	4	0.125
078	16	>128	8	4	64	0.125
106	16	128	8	8	4	0.06
126	16	64	16	>64	4	0.125
356	32	>128	8	8	4	0.03
356	32	>128	8	8	8	0.06
356	32	>128	8	8	4	0.25
356	>64	128	8	16	4	0.25

Table 2 MCB3681 MICs (mg/L) in C. difficile isolates with dual quinolone-oxazolidinone resistance (highlighting indicates resistance). Clindamycin and chloramphenicol MICs[4] are also shown.

MXF=moxifloxacin; CIP=ciprofloxacin; LZD=linezolid; CLI=clindamycin; CHL=chloramphenicol