Novel pharmacology and susceptibility of ibezapolstat against *Clostridioides difficile* isolates with reduced susceptibility to *C.difficile* directed antibiotics

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ABSTRACT

Background: Ibezapolstat is a Gram-positive selective spectrum antibiotic in phase 2 clinical trials for *C. difficile* infection. With a unique mechanism of action that targets the DNA pol IIIC enzyme preferentially upregulating genes near the origin of replication, IBZ should maintain activity against *C. difficile* with reduced susceptibility to other CDI-directed antibiotics and may demonstrate unique pharmacologic properties. The goal of this study was to assess the susceptibility of IBZ against strains with reduced susceptibility to current CDI antibiotics and assess motility inhibition.

Methods: Agar dilution MIC studies were performed against *C. difficile* strains with reduced susceptibility to metronidazole, vancomycin, and fidaxomicin following CLSI document M11-A7 for anaerobic bacteria. *C.difficile* motility was assessed using a phenotypic motility assay and quantitative RT-PCR vs. relevant flagellar genes (fliA, flgb, fliC) using *C. difficile* strain CD630 pre-treated with sub-MIC of IBZ adapted from the methodology of Doan *et al*. (Antibiotics 2022).

Results: Twelve isolates with reduced susceptibility to metronidazole (MIC range: 0.25-8 ug/mL), vancomycin (MIC range: 1-16 ug/mL) or fidaxomicin (<0.03125-2 ug/mL) were tested. IBZ MIC50 and MIC90 did not differ between susceptible and reduced susceptible isolates. MBC values did not differ between wild-type and reduced susceptible isolates with values similar to results with vancomycin using wild-type strains. Motility assay demonstrated reduced *C. difficile* movement in agar with pre-treatment with sub-MIC IBZ and reduction in flagellar gene expression with sub-MIC IBZ exposure.

Conclusion: IBZ maintain activity against *C. difficile* strains with reduced susceptibility to metronida-zole, vancomycin, and fidaxomicin. A novel pharmacologic property of IBZ was identified likely due to its unique mechanism of action. These findings support the continued clinical development of IBZ.

BACKGROUND

Ibezapolstat (IBZ)

- Gram-positive selective spectrum (GPSS) antibiotic, unique mechanism of action targets pol IIIC DNA polymerase
- Potent activity against *C. difficile*
- Completed Phase IIa CDI clinical trial: 100% success rate with favourable microbiome changes
- Currently in a phase IIb clinical trial

OBJECTIVES

- To assess *in vitro* efficacy of ibezapolstat against selected isolates with reduced susceptibility to currently used CDI antibiotics.
- Measure the effect of ibezapolstat on *C. difficile* motility and flagellar genes.

RESULTS

Table 1. Ibezapolstat efficacy against selected clinical isolates

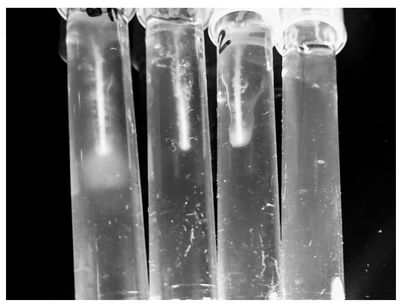
IBZ Retained Activity vs. Resistant Strains

	metronidazole	vancomycin	fidaxomicin	ibezapolstat
MT 4802	0.25	1	0.006	4
MT 5529	2	1	0.5	4
SH 1132	1	4	0.5	8
MT 5342	2	8	1	4
MT 5364	2	4	1	4
MT 5426	4	4	0.5	8
MT 5515	4	4	2	8
MT 4883	2	8	2	8
MT 5493	2	1	1	4
MT 5536	0.25	2	1	8
MT 5382	2	1	1	8
MT 5071	1	4	1	8

- MICs in mg/L
- MIC values associated with susceptibility against an antibiotic with reduced susceptibility (difference >8xMIC value)

Figure 1. Motility assay

IBZ Reduced *C. difficile* Motility

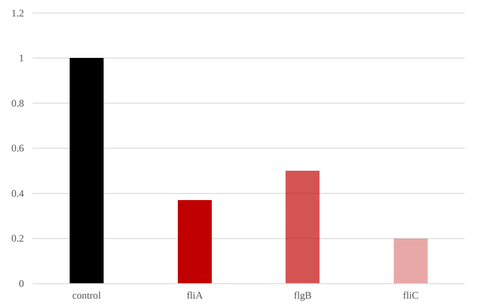


\- 1 2 4 Ibezapolstat (mg/L)

- Reference strain CD630 treated with sub-inhibitory to inhibitory concentrations of ibezapolstat in semi-solid BHI agar for 3 days

Figure 2. Flagellar gene expression

IBZ Decreased Flagellar Genes



- Relative expression of CD 630 strain's flagellar genes
- 4h treatment with sub-inhibitory concentration of ibezapolstat (0.5xMIC)
- 2-to-5-fold decrease of flagellar genes observed compared to control (gluD)

METHODS

MIC testing

Cultures of *C. difficile* were prepared by inoculating one colony to Brain Heart Infusion broth (BHI) supplemented with 0.1% sodium taurocholate. After 24h incubation at 37°C in anaerobic chamber, pre-cultures were diluted 1:100 to approximately 10^6CFU/mL in fresh BHI and the appropriate concentration of antibiotic. Inhibitory concentra-tions were determined by eye visualization at 24h.

Motility assay

Bacterial strains were inoculated in BHI medium containing 0.3% agar at sub-inhibitory concentra-tions of antibiotic and grown anaerobically at 37°C for 3 days.

Expression of flagellar genes

Bacterial pre-cultures were diluted, then grown in BHI at sub-inhibitory concentrations of antibiotic at 37°C under anaerobic conditions for 4h and transcripts levels of *fliA*, *fliB* and *fliC* were measured by qRT-PCR (Doan *et al.* 2022).

CONCLUSIONS

- Ibezapolstat maintains its efficacy against clinical isolates with reduced susceptibility to metronidazole, vancomycin, and fidaxomicin.

Unexpected, Novel Findings

- Ibezapolstat reduces *C. difficile's* motility as visualized with CD 630 reference strain in a motility assay.
- Motility inhibition has been quantified by measuring the relative expression of flagellar genes fliA, B and C.
- Future work will explore bacterial motility and adherence to epithelial cells and biofilms.
- These results support continued clinical development of ibezapolstat and further elucidate the mechanism of action of DNA polymerase inhibitors.

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