Persistence and removal of fidaxomicin from *C. difficile* spores, and effects on recovery

P0799

Chilton CH[1], Ashwin H[1], Crowther GS[1], Longshaw C[2], and Wilcox MH[1,3].

[1] Leeds Institute for Biomedical and Clinical Sciences, University of Leeds, Leeds, UK [2] Astellas Pharma Europe Ltd, 2000 Hillswood Drive, Chertsey, Surrey, UK. [3] Microbiology Department, Leeds Teaching Hospitals NHS Trust, Leeds, UK

Introduction

Clostridium difficile infection (CDI) continues to be a leading cause of antibiotic associated diarrhoea[1] and a major burden on healthcare facilities worldwide.[2,3] Treatment options are limited and are associated with high rates of recurrence (~20%).[4] Fidaxomicin, a narrow spectrum macrocyclic antimicrobial with good activity against *C. difficile*, was recently approved for CDI treatment. In phase III clinical trials fidaxomicin was non-inferior to vancomycin for initial clinical cure of CDI, but significantly reduced the rate of recurrent infection.[5]

We have previously reported the persistence of detectable fidaxomicin activity within an *in-vitro* human gut model for a prolonged duration compared with vancomycin.[6] For much of this time, spores were not detected, a phenomenon not observed following vancomycin treatment. We have also noted the persistence of fidaxomicin (but not vancomycin) activity on *C. difficile* spores following washing, and within gut model biofilm structures.[7] Here we have investigated the effect of wash buffer on persistence of antimicrobial activity on *C. difficile* spores, and subsequent spore recovery.

Methods

Fidaxomicin adherence to spores

C. difficile spores of PCR ribotype 001 were exposed to 200 mg/L fidaxomicin, or non-antimicrobial containing control solutions for 30 mins at 37°C. One ml aliquots were centrifuged and washed three times in 500 µl of either phosphate buffered saline (PBS), ethanol, dimethyl sulfoxide (DMSO) or cetyltrimethylammonium bromide (CTAB). Samples were then re-suspended in PBS, transferred to fresh tubes and rinsed twice in PBS to remove residual wash solution.

The persistence of antimicrobial activity was evaluated by large plate bioassays using *Kocuria rhizophila* (ATCC 9341) and *Staphylococcus aureus* indicator organisms. Sterilised molten Wilkins-Chalgren agar was inoculated with 1 ml of indicator organism. Holes were made in bioassay plates with a cork borer and 20 µL samples added to holes. After incubation for 24h at 37 °C, zones of inhibition were measured with callipers. Antimicrobial activity was determined against calibration series (2-128 mg/L) of fidaxomicin (*K. rhizophila* containing plates) and vancomycin (*S. aureus* containing plates).

Recovery of washed spores was determined by serial dilution (10-fold) in PBS, followed by culture on Brazier's CCEYL agar.

Results

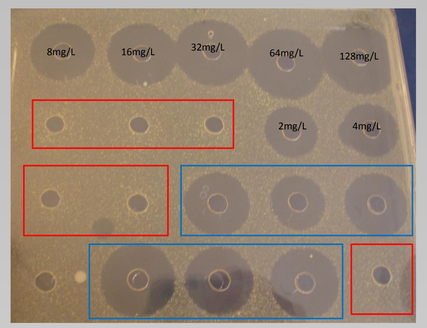

Figure 1. Bioassay of Fidaxomicin calibrants and washed spores.

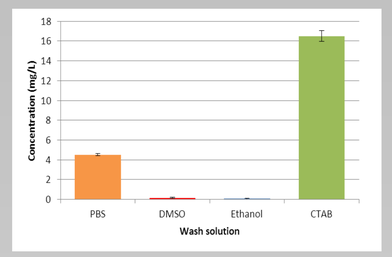

Figure 2. Concentration of fidaxomicin on spores washed in different buffers.

Zones of inhibition were visible for PBS and CTAB washed spores (Figure 1, blue boxes), whereas no zones were visible for ethanol or DMSO washed spores (Figure 1, red boxes).

The concentration of antimicrobial persisting (average of 6 replicates) following PBS washing was found to be ~4 mg/L (Figure 2), similar to previously reported levels.

CTAB washed spores retained greater fidaxomicin activity (~16 mg/L; 4-fold greater) than PBS washed spores (Figure 2).

		Aliquots (n)	Inhibition of recovery (Fidaxomicin-exposed)
	Initial spore preparation	3	Neat, 1:10, 1:100
Wash	PBS	6	Neat
	DMSO	6	None
	Ethanol	6	None
	CTAB	6	Neat, 1:10, 1:100

Table 1. Levels of inhibition of spore recovery from fidaxomicin-exposed spore preps after washing.

Inhibition of spore recovery was observed for unwashed, PBS washed and CTAB washed spores, but not ethanol or DMSO washed spores (Table 1).

This inhibition was removed by dilution.

With greater concentrations of fidaxomicin measured on spore preparations, recovery of spores was inhibited for longer. Thus, recovery of CTAB washed spores was inhibited at 1:100 dilution, whereas recovery of PBS washed spores was inhibited in neat samples only.

			Spore count (log_{10} cfu/mL)	
		Aliquots (n)	Non-fidaxomicin-exposed	Fidaxomicin-exposed
	Initial spore preparation	3	7.38	8.97
Wash	PBS	6	5.85	7.78
	DMSO	6	3.20	3.59
	Ethanol	6	6.30	6.61
	CTAB	6	9.56	9.34

Table 2. Viable counts for spore preparations and washed spore aliquots.

Once inhibition of recovery was removed, enumeration of non-fidaxomicin exposed and fidaxomicin-exposed spores was similar (Table 2), although varied with wash solvent.

Recovery of DMSO washed spores was lower than other wash buffers

Interestingly, washing with CTAB increased recovery of spores compared with initial spore preparations.

Discussion

Choice of wash buffer affected fidaxomicin persistence on spores and subsequent spore recovery on CCEYL agar.

Ethanol and DMSO removed fidaxomicin activity from spores, whereas PBS and CTAB did not. This may be due to solvent polarity. Fidaxomicin does not dissolve readily in water, but is soluble in DMSO.

When antimicrobial activity was retained on spores, recovery of spores was inhibited (Table 1*)*. Inhibition was removed by dilution, and correlated with antimicrobial concentrations measured by bioassay. It may be that the amount of fidaxomicin is insufficient to adhere to all spores, and that dilution allows non-coated spores to germinate, whereas in concentrated solutions they are inhibited by neighbouring fidaxomicin-bound spores.

Wash solvent affected spore recovery independently of fidaxomicin. The density of solvent will affect pelleting of spores during centrifugation, thus affecting recovery. DMSO, a relatively dense solvent, had the greatest reduction on spore recovery (~40%).

The reason for increase in spore recovery following CTAB washing is unclear, but may be due to surfactant properties. It is possible that surfactants interrupt spore coats in some way, helping to break spore dormancy, thereby increasing yield on CCEYL.

Conclusions

This work provides further evidence of the existence and nature of fidaxomicin persistence on *C. difficile* spores.

The extent of fidaxomicin persistence on washed *C. difficile* spores is dependent on the choice of wash solution, which may relate to solvent polarity.

DMSO and ethanol removed spore-associated fidaxomicin activity, whereas PBS and CTAB did not.

References

1. Freeman J, Bauer MP, Baines SD, *et al*. *Clin Microbiol Rev* 2010; **23**: 529.
2. Wiegand PN, Nathwani D, Wilcox MH, *et al*. *J Hosp Infect* 10-4-2012;
3. Ghantoji SS, Sail K, Lairson DR, *et al*. *J Hosp Infect* 2010; **74**: 309-318.
4. Johnson S. *J Infect* 2009; **58**: 403-410.
5. Louie TJ, Miller MA, Mullane KM, *et al*. *New Engl J Med* 2011; **364**: 422-431.
6. Chilton CH, Crowther GS, Feeman J *et al. J Antimicrob Chermother,* 2014; **69**:451-62
7. Chilton CH,, Crowther GS, Todhunter SL *et al*. ECCMID 2013; poster LB-2817

Acknowledgements

This study was financially supported by Astellas Pharma Europe Ltd

Contact

mark.wilcox@leedsth.nhs.uk
c.h.chilton@leeds.ac.uk