Journal of Antimicrobial Chemotherapy Advance Access published February 11, 2009

Journal of Antimicrobial Chemotherapy
doi:10.1093/jac/dkp017

JAC

Mecillinam: a low-risk antimicrobial agent for induction of *Clostridium difficile* infection in an *in vitro* human gut model

Simon D. Baines[1], Rachael O'Connor[1], Grace Huscroft[1], Katie Saxton[1], Jane Freeman[2] and Mark H. Wilcox[1,2]*

[1]*Department of Microbiology, Institute of Molecular and Cellular Biology, University of Leeds, Leeds LS2 9JT, UK;* [2]*Department of Microbiology, The General Infirmary, Old Medical School, Leeds LS1 3EX, UK*

Keywords: amidino-penicillin, spores, cytotoxin

*Corresponding author. Department of Microbiology, The General Infirmary, Old Medical School, Leeds LS1 3EX, UK. Tel: +44-113-3926818; Fax: +44-113-3435649; E-mail: Mark.Wilcox@Leedsth.nhs.uk

Sir,

Clostridium difficile infection (CDI) is a major cause of diarrhoea among the hospitalized elderly and a significant financial burden upon healthcare systems worldwide.[1] Incidence continues to increase, particularly following the emergence of hypervirulent *C. difficile* PCR ribotype 027 (NAP1/BI).[2] CDI is thought to arise as a result of depletion of the colonic microflora and colonization resistance by broad-spectrum antibiotics,[3] allowing *C. difficile* germination, proliferation and toxin production. Antimicrobials differ markedly in their propensity to induce CDI both *in vitro* and *in vivo*. Piperacillin/tazobactam has a broad spectrum of activity but is considered low risk for CDI induction. In contrast, third-generation cephalosporins are relatively high risk for CDI but have narrower spectra of activity.[3]

Mecillinam is an amidino-penicillin β-lactam agent, used mainly for the treatment of urinary tract infections. It is principally active against Enterobacteriaceae, but is poorly active against Gram-positive bacteria. Few data exist on the propensity of mecillinam to induce CDI *in vivo*.[4] We examined the effect of mecillinam on two epidemic *C. difficile* strains (PCR ribotypes 001 and 027) and the indigenous gut microflora using a triple-stage chemostat human gut model. We have previously used the gut model to investigate both antimicrobial induction of and therapeutic interventions for CDI,[5] and results correlate well with clinical observations. However, although this is a reproducible model of CDI, it cannot simulate secretory or immunological events, which may be important in *C. difficile* pathogenesis.

The model was prepared and inoculated, and *C. difficile* total counts, spores and toxin titres and gut bacterial populations were monitored as described previously. Gut bacterial populations were allowed to equilibrate (14 days), whereupon $\sim 10^7$ cfu of *C. difficile* spores was added. No further interventions were made for 7 days. A further $\sim 10^7$ cfu of *C. difficile* spores was added and mecillinam instillation commenced (1 mg/L mecillinam twice-daily for 7 days) to reflect published faecal concentrations.[4] No further interventions were made. Mecillinam concentrations were determined by large-plate bioassay using *Escherichia coli* ATCC 25922 as described previously.[4] MICs of mecillinam for 60 *C. difficile* isolates were measured using a reference agar dilution method (CLSI) for anaerobe susceptibility testing.

In the absence of mecillinam, gut bacterial populations remained stable, with no evidence of *C. difficile* spore germination, proliferation or cytotoxin production in either experiment, as reported previously (Figure 1a and b).[5] Similarly, *C. difficile* spores remained quiescent during and after mecillinam instillation (Figure 1a). Behaviours of both *C. difficile* ribotypes were similar, demonstrating no detectable cytotoxin (PCR ribotype 027) or ≤2 relative units (RU) (PCR ribotype 001) following the dosing period (Figure 1a, period C). We consider the latter low cytotoxin titres to be of doubtful significance given the absence of sustained *C. difficile* proliferation in an epidemic strain with a recognized propensity to induce CDI *in vivo*.[5] Prior gut model experiments have indicated that subinhibitory concentrations of some antimicrobials (e.g. clindamycin and cefotaxime) elicit *C. difficile* germination, proliferation and high-level cytotoxin production.[5] Mecillinam concentrations peaked at 2.7 mg/L (ribotype 001) and 3.6 mg/L (ribotype 027), >100-fold less than the MIC (>128 mg/L) for all isolates tested. Germination, proliferation and high-level (≥4 RU) cytotoxin production were not observed, indicating that induction of *C. difficile* proliferation and high-level cytotoxin production in the gut model may be antimicrobial-specific. Mecillinam had little effect on most enumerated bacterial groups in the gut model in agreement with *in vivo* studies.[4] *Bacteroides fragilis* group and *Bifidobacterium* spp. were most numerous. Mecillinam instillation precipitated a transient decline in *Bifidobacterium* spp. and a gradual decline in lactose fermenters. This may be accounted for by the pharmacodynamics of the drug, which can be affected by the growth phase and bacterial inoculum density.[4]

Our results suggest that exposure of epidemic *C. difficile* strains to concentrations of mecillinam found in the human gut following oral dosing is not a significant risk factor for CDI induction. This may be explained by at least two factors. First, there was minimal gut microflora disruption in the presence of *in vivo* mecillinam concentrations and therefore little reduction in colonization resistance. While no consistent changes in bacterial genera have been correlated with simulated CDI in the gut model, a change from the predominance of obligate anaerobes (*B. fragilis* group and bifidobacteria) to that of facultative anaerobes was observed in previous studies where high-level cytotoxin production was observed.[5] Mecillinam did not precipitate such changes. Second, mecillinam may not stimulate *C. difficile* directly. We postulated that antimicrobial concentrations may be important in determining the timing of *C. difficile* growth and cytotoxin production, possibly influencing *C. difficile* behaviour directly.[5] Recent research indicates that antimicrobials may directly up-regulate gene expression to a greater (clindamycin and amoxicillin) or lesser (metronidazole) extent.[6] Mecillinam may not up-regulate *C. difficile* gene expression. Future use of quantitative molecular technologies may help elucidate the behaviour of *C. difficile* exposed to antimicrobial stresses, and

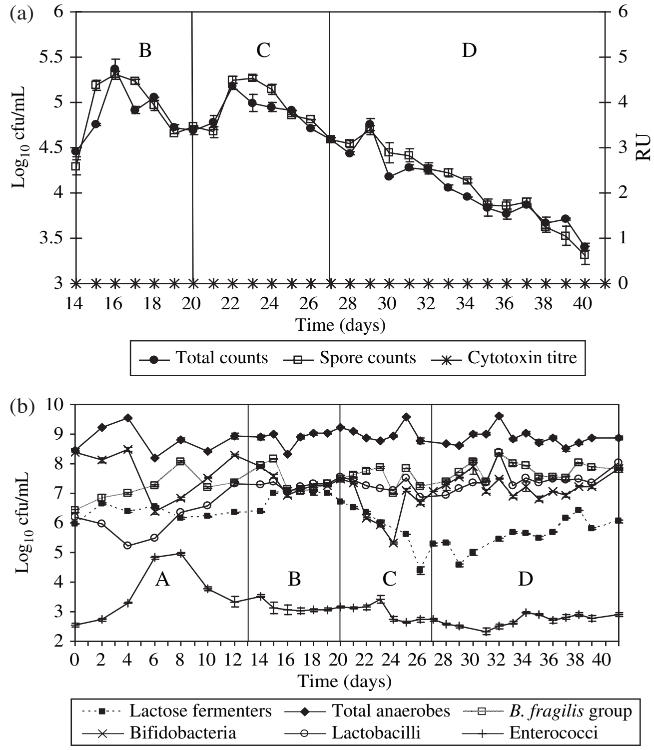

Figure 1. Mean (±SE) (a) *C. difficile* total counts and spore counts ($\log_{10}$ cfu/mL) and cytotoxin titres ($\log_{10}$ RU) and (b) indigenous gut microflora ($\log_{10}$ cfu/mL) in vessel 3 of the *C. difficile* ribotype 027 gut model. Vertical lines indicate the final day of each experimental time period.

so help to identify bacterial groups potentially important in colonization resistance. Overall, we believe this study indicates that mecillinam likely has a low propensity to induce CDI.

Acknowledgements

We thank Dr Warren Nigel Fawley for performing PCR ribotyping of the *C. difficile* strains used in this study.

Funding

This work was supported by a research grant from LEO Pharma.

Transparency declarations

M. H. W. has received honoraria for consultancy work, financial support to attend meetings and research funding from Astra-Zeneca, Bayer, Genzyme, Nabriva, Novacta, Pfizer, Targanta Therapeutics and Wyeth. S. D. B. has received financial support to attend meetings from Bayer and Targanta Therapeutics. K. S. has received financial support to attend meetings from Bayer. J. F. has received financial support to attend meetings from Bayer and Wyeth. G. H. and R. O'C. have nothing to declare.

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