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Note

Susceptibility of *Clostridium* species isolated in Japan to fidaxomicin and its major metabolite OP-1118

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ABSTRACT

The narrow-spectrum macrocyclic antibiotic fidaxomicin is approved for treatment of *Clostridium difficile* infection in many countries and is currently under evaluation in Japan for this indication. This study was conducted to evaluate the effects of fidaxomicin and its major metabolite, OP-1118, on *Clostridium* spp. isolated in Nagasaki University Hospital, Japan. Isolates were cultured and antimicrobial susceptibility analyses performed according to the Clinical Laboratory Standards Institute methods.

Ninety-eight isolates were obtained between 2012 and 2015, 50 of *C. difficile* and 48 of eight other *Clostridium* spp. Fidaxomicin had the lowest minimum inhibitory concentration (MIC) of the antimicrobials tested against *C. difficile*, with MIC_{90} (MIC range) 0.12 μg/mL (0.015–0.25), versus vancomycin MIC_{90} 0.5 μg/mL (0.5), metronidazole MIC_{90} 0.5 μg/mL (0.12–0.5), and OP-1118 MIC_{90} 4.0 μg/mL (0.5–4.0). Fidaxomicin and OP-1118 each had a similar spectrum of activity against the other *Clostridium* spp. *C. butyricum* and the 29 fidaxomicin- and OP-1118-susceptible *C. perfringens* isolates had the lowest MIC values, and *C. bolteae* and *C. hathewayi* higher. All the *C. ramosum* isolates (n = 6) and one of 30 *C. perfringens* isolates had low susceptibility to fidaxomicin and OP-1118 (i.e., MIC >64 μg/mL).

In summary, this study showed that fidaxomicin was active against a number of *Clostridium* spp., including *C. difficile*. Fidaxomicin was generally more effective than its major metabolite OP-1118, but both showed a similar spectrum of activity, suggesting that OP-1118 contributes to the antimicrobial activity of fidaxomicin. These findings were broadly in accordance with those of similar studies conducted in other settings.

Clostridium difficile (reclassified as *Clostridioides difficile*) infection (CDI) occurs worldwide, including in Asia, and is often found in hospitals and long-term care facilities [1]. Older patients recently treated with broad-spectrum antibiotics, which deplete the natural intestinal microbiota, are at the highest risk of infection. Treatment is typically with antimicrobials such as vancomycin and metronidazole [1]. The efficacy of the narrow-spectrum macrocyclic antibiotic fidaxomicin for the treatment of CDI was demonstrated in Phase III clinical trials in North America and Europe. In these studies, it was shown to be non-inferior to vancomycin for initial cure of CDI, but associated with significantly reduced rates of disease recurrence compared with vancomycin [2,3]. Based on these findings, fidaxomicin has been approved in a number of countries for treatment of CDI in adults. Fidaxomicin is bactericidal against *Clostridium difficile*, including against so-called "hypervirulent" restriction endonuclease type BI and PCR-ribotype 027 strains [4]. Fidaxomicin, however, lacks activity against commonly cultured Gram-negative commensal bacteria in the colon (e.g., *Bacteroides* spp.) and has relatively little effect on Gram-positive commensal bacteria, including Clostridial cluster XIVa and IV species such as *C. coccoides* and *C. cellulosi*, respectively [5–7]; it therefore has minimal effects on the natural microbiota thought to be important for *C. difficile* colonization resistance [5,6,8,9]. An *in vitro* study of isolates of indigenous bowel microbiota, conducted in the United States, compared the activity of fidaxomicin with other antimicrobial

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agents; it was found that the *Clostridium* spp. sensitive to fidaxomicin were mostly members of the Clostridial phylogenetic clusters I and XI, for example, *C. difficile*, *C. perfringens*, *C. bifermentans* (reclassified as *Paraclostridium bifermentans*), and *C. glycolicum* (reclassified as *Terrisporobacter glycolicus*) [9].

In a Phase I study, fidaxomicin demonstrated similarly minimal systemic absorption, and was mainly excreted in feces in Japanese and Caucasian healthy volunteers, with a good safety and tolerability profile [10]. Following oral administration (400 mg per day), fidaxomicin is excreted mainly in the feces along with high concentrations of its major metabolite, OP-1118, which is derived by hydrolysis of the isobutyryl ester located at the 4′ position of fidaxomicin [4]. Similar to the parental compound, OP-1118 is poorly absorbed and is bactericidal against *C. difficile* strains at concentrations that are several orders of magnitude below the detected fecal concentrations of this compound after oral administration of fidaxomicin [4,10]. The efficacy and safety of fidaxomicin in Japanese patients is being evaluated in a Phase III clinical trial (NCT02179658). As yet, however, there are no data on the susceptibility of *Clostridium* spp. likely to be present in isolates from a hospital setting in Japan to fidaxomicin and OP-1118. Therefore, this study was conducted to evaluate the effects of fidaxomicin and OP-1118 on *Clostridium* spp. isolated in a large hospital in Japan.

Clostridium spp. were isolated at Nagasaki University Hospital between February 2012 and January 2015. Samples were stored at −70 °C or below in Microbank™ cryogenic vials (Pro-lab Diagnostics, Round Rock, TX, USA). Test strains were then thawed and immediately plated on Anaero Columbia agar supplemented with rabbit blood (Nippon Becton Dickinson Co. Ltd, Tokyo, Japan), then incubated under anaerobic conditions for 24–48 h at 35 °C. Fresh cultures were suspended in Gifu Anaerobic Medium (GAM) broth (Nissui Pharmaceutical Co. Ltd, Tokyo, Japan) to a turbidity of 0.5 McFarland (approximately $1–2 \times 10^8$ CFU/mL). After further dilution in GAM broth, a final 5 μL inoculum of approximately $1–2 \times 10^5$ CFU/spot was used for assays.

Susceptibility assays were performed according to Clinical Laboratory Standards Institute (CLSI)-approved standards for anaerobes (M11-A8, M100-S25) [11,12] using Brucella agar (Nippon Becton Dickinson Co Ltd, Tokyo, Japan) supplemented with hemin (5 μg/mL), vitamin K1 (1 μg/mL; both from Sigma-Aldrich Japan Co. LLC), and pooled laked sheep blood (5% v/v; LSB, Nippon Bio-Supp. Center, Tokyo, Japan). The medium (9 vol) was mixed with 1 volume of fidaxomicin or OP-1118 (Astellas Pharma, Inc., Japan) diluted in 0.1 mol/L phosphate buffer solution (pH 8.0). The final concentrations of fidaxomicin or OP-1118 in the prepared media were within the ranges of 0.004–64 μg/mL. Sterile distilled water was used instead of the study drugs for the control medium.

A quality control (QC) strain, *C. difficile* ATCC® 700057™, was prepared and evaluated in parallel with the study isolates. This strain had a minimum inhibitory concentration (MIC) range for fidaxomicin of 0.06–0.25 μg/mL [12]. As there are currently no CLSI criteria for OP-1118, a MIC range of 0.25–2 μg/mL was established by assaying the MIC for the QC strain in triplicate.

Ninety-eight *Clostridium* spp. isolated in Nagasaki University Hospital were used for evaluation. These included 50 *C. difficile* strain isolates (Fig. 1 and Table 1) and 48 isolates of eight other *Clostridium* spp. (Table 1).

Both fidaxomicin and OP-1118 were active against all of the *C. difficile* strains studied, with fidaxomicin having the lowest MIC of the antimicrobials tested (Fig. 1). Fidaxomicin had a MIC at which 90% of isolates were inhibited (MIC_{90}) of 0.12 μg/mL (MIC range 0.015–0.25 μg/mL), compared with 4.0 μg/mL for OP-1118 (MIC range 0.5–4.0 μg/mL) (Fig. 1 and Table 1). The MIC_{90} for vancomycin and metronidazole, respectively, were 0.5 μg/mL (0.5) and 0.5 μg/mL (0.12–0.5). The MIC values for the *C. difficile* QC strain for both agents were within acceptable QC ranges (fidaxomicin: all 0.06 μg/mL; OP-1118: range 0.5–1 μg/mL).

Fidaxomicin and OP-1118 also had a similar spectrum of antimicrobial activity against the other *Clostridium* spp. tested (Table 1). Among *Clostridium* spp. isolates, some differences in susceptibility were observed: the lowest MICs were observed with *C. butyricum* and the fidaxomicin- and OP-1118-susceptible *C. perfringens* isolates, and the highest MICs with *C. bolteae* and *C. hathewayi* (reclassified as *Hungatella hathewayi*) (Table 1). All *C. ramosum* isolates (n = 6) and only one of the 30 *C. perfringens* isolates had low susceptibility to fidaxomicin and OP-1118 (i.e., MIC >64 μg/mL) (Table 1). Both fidaxomicin and OP-1118 demonstrated more potent antimicrobial activity against Clostridial clusters I and XI, and less potent activity against XIVa and XVIII (Table 1); this was in line with previous publications [9].

The observations in this study of a slightly higher MIC for OP-1118 than for fidaxomicin are in accordance with previous findings for *C. difficile* [4]. A number of studies have included evaluation of the bactericidal activity of fidaxomicin against isolates of some of the same *Clostridium* spp. assessed in the present study [9,13–15]. These other studies reported broadly similar patterns of

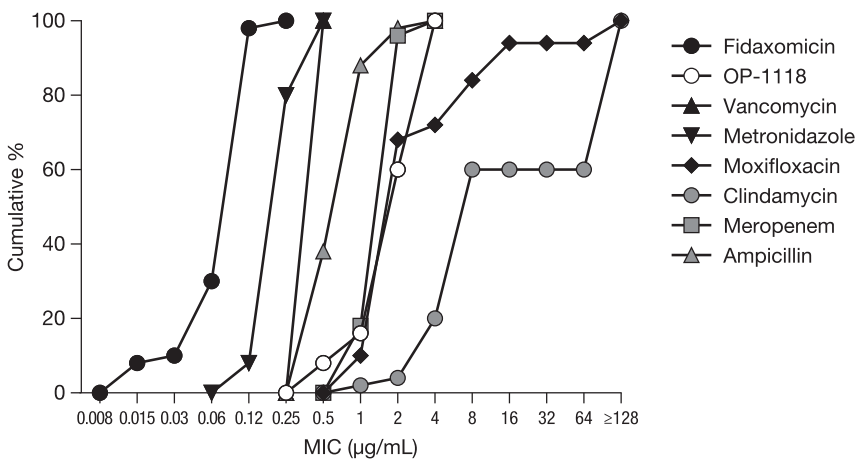

Fig. 1. The cumulative minimum inhibitory concentration distributions of fidaxomicin, its metabolite OP-1118 and other antimicrobials against 50 *Clostridium difficile*[a] isolates. MIC, minimum inhibitory concentration. [a]Reclassified as *Clostridioides difficile*.

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