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In vitro activity of XF-73, a novel antibacterial agent, against antibiotic-sensitive and -resistant Gram-positive and Gram-negative bacterial species

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ABSTRACT

The antibacterial activity of XF-73, a dicationic porphyrin drug, was investigated against a range of Gram-positive and Gram-negative bacteria with known antibiotic resistance profiles, including resistance to cell wall synthesis, protein synthesis, and DNA and RNA synthesis inhibitors as well as cell membrane-active antibiotics. Antibiotic-sensitive strains for each of the bacterial species tested were also included for comparison purposes. XF-73 was active [minimum inhibitory concentration (MIC) 0.25–4 mg/L] against all of the Gram-positive bacteria tested, irrespective of the antibiotic resistance profile of the isolates, suggesting that the mechanism of action of XF-73 is unique compared with the major antibiotic classes. Gram-negative activity was lower (MIC 1 mg/L to >64 mg/L). Minimum bactericidal concentration data confirmed that the activity of XF-73 was bactericidal. Time–kill kinetics against healthcare-associated and community-associated meticillin-resistant *Staphylococcus aureus* isolates demonstrated that XF-73 was rapidly bactericidal, with >5 $\log_{10}$ kill obtained after 15 min at 2× MIC, the earliest time point sampled. The post-antibiotic effect (PAE) for XF-73 under conditions where the PAE for vancomycin was <0.4 h was found to be >5.4 h. XF-73 represents a novel broad-spectrum Gram-positive antibacterial drug with potentially beneficial characteristics for the treatment and prevention of Gram-positive bacterial infections.

1. Introduction

The impact of antibiotic-resistant bacteria on the treatment of infections continues its relentless march upwards. Meticillin-resistant *Staphylococcus aureus* (MRSA) is the prime example; MRSA is increasingly prevalent throughout the world and is endemic in some hospital and community settings [1–3]. Healthcare-associated MRSA (HA-MRSA) now accounts for 40% of *S. aureus* infections in the UK and 63% of *S. aureus* infections in the USA [1,2].

Community-associated MRSA (CA-MRSA) is also becoming more widespread; in the USA, 59% of skin and soft-tissue infections (SSTIs) presenting to emergency departments are caused by CA-MRSA [3]. The most prevalent CA-MRSA clone in the USA, USA300, which carries the staphylococcal cassette chromosome *mec* (SCC*mec*) type IVa and Panton–Valentine leukocidin genes, now accounts for 61–87% of MRSA isolates from SSTIs in North American emergency departments [4,5] and is also emerging as a serious cause of healthcare-associated infections [6–8].

A further complication is the emergence of multidrug-resistant (MDR) bacteria both in Gram-positive and Gram-negative species. There is a critical need for new antibacterials, particularly those with activity against MDR bacteria.

XF-73 is a dicationic porphyrin drug and previous studies have shown that XF-73 has potent bactericidal activity [minimum bactericidal concentration for 90% of organisms (MBC_{90}) = 0.5 mg/L] in vitro against a range of clinically important *S. aureus* isolates, including meticillin-sensitive *S. aureus* (MSSA), HA-MRSA and CA-MRSA [9]. The drug is being developed to treat *S. aureus* and MRSA infections, and clinical trials are underway to assess the potential of XF-73 for nasal decolonisation of *S. aureus*. Investigations into the mechanism of action of XF-73 against *S. aureus* have demonstrated that the drug exhibits a rapid cell membrane-perturbing activity that is likely to be responsible for inhibition of macromolecular synthesis and death of staphylococci exposed to the drug [10]. During prolonged laboratory subculture (55 passages) in the presence of sub-minimum inhibitory concentrations (sub-MICs) of XF-73, no resistant mutants were generated using five Network on Antimicrobial Resistance in *Staphylococcus aureus* (NARSA) [11] strains of MRSA [9,12].

The purpose of this study was (i) to examine the breadth of in vitro activity of XF-73, using broth microdilution, against a range of antibiotic-sensitive and -resistant Gram-positive and

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Gram-negative aerobic and anaerobic bacterial species and (ii) to investigate the effect of existing antibiotic resistance mechanisms on the activity of XF-73. Strains in the screening panel were chosen to include examples of bacteria with all known antibacterial resistance mechanisms. Comparator antibiotics were included to confirm the expected MICs for reference strains and to confirm that the MICs for clinical isolates were within the expected ranges, confirming the relevance of selection of these clinical isolates. Time–kill kinetics using *S. aureus* were also investigated to determine the speed of the bactericidal activity of XF-73. Determination of the post-antibiotic effect (PAE) for *S. aureus* of XF-73 for a range of different multiples of the MIC was also undertaken and compared with a comparator antibiotic.

2. Materials and methods

2.1. Bacterial strains

A panel of 101 Gram-positive and Gram-negative organisms, including anaerobes, was selected for susceptibility and cross-resistance testing (Table 1; Supplementary Table 1). The panel comprised 65 Gram-positive isolates (56 aerobic and 9 anaerobic) and 36 Gram-negative isolates (31 aerobic and 5 anaerobic). Examples of isolates resistant to cell wall inhibitors (β-lactams, glycopeptides and cephalosporins), protein synthesis inhibitors (oxazolidinones, macrolides and mupirocin), DNA synthesis inhibitors (fluoroquinolones), RNA synthesis inhibitors (rifampicin) and bacterial cell membrane-active antibiotics (daptomycin) were included in the panel.

The Gram-positive panel (Table 1; Supplementary Table 1) included: MRSA (control and clinical isolates, including a linezolid-resistant isolate); MSSA; vancomycin-intermediate-resistant *S. aureus* (VISA); high-level mupirocin-resistant (MIC > 256 mg/L) and daptomycin-non-susceptible (MIC = 2 mg/L) clones of *S. aureus*; antibiotic-susceptible and meticillin-resistant *Staphylococcus epidermidis*; antibiotic-susceptible and penicillin- and/or macrolide-resistant *Streptococcus* spp.; vancomycin-resistant (*vanA*, *vanB* and *vanC*) *Enterococcus* spp.; and toxin-positive and ceftriaxone-resistant strains of *Clostridium difficile*.

The Gram-negative panel (Table 1; Supplementary Table 1) included MDR strains of *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*.

Clinical and Laboratory Standards Institute (CLSI) antimicrobial susceptibility control strains were included in the screen to confirm that the expected MICs were obtained for the comparator compounds (vancomycin, linezolid and clindamycin) and, where possible, an antibiotic-sensitive and an antibiotic-resistant isolate was included for all species tested.

For investigation of killing kinetics and determination of the PAE, two NARSA [11] strains were used, comprising one CA-MRSA (NRS123) and one HA-MRSA (NRS382).

2.2. Antimicrobial agents

XF-73 was obtained from Destiny Pharma Ltd. (Brighton, UK). Vancomycin (batch number 016K1102) and fusidic acid (batch number 065K1404) were obtained from Sigma-Aldrich Ltd. (Gillingham, UK), and mupirocin (batch number B143832) was obtained from Mast Diagnostics (Bootle, UK).

2.3. Susceptibility testing

MICs were determined by the broth microdilution method according to approved M7-A7 (aerobes) and M11-A7 (anaerobes) standards of the CLSI [13,14]. MIC values were read after 24 h or 48 h of incubation, depending on the species.

MBC values were determined by subculturing wells showing no visible growth onto pre-labelled and quartered Columbia blood agar plates. Plates were incubated appropriate to the species and the number of surviving bacterial colonies was counted. MBC values were determined for each isolate as the antimicrobial concentration producing a $3\log_{10}$ drop (99.9%) in bacterial colony-forming units (CFU)/mL compared with the original inoculum, based on a 5% error for determination of final inoculum [15].

2.4. Media used

Mueller–Hinton (MH) broth (Oxoid Ltd., Basingstoke, UK) was used to prepare the inoculum and according to CLSI requirements [13,14]. For fastidious isolates, MH broth was supplemented with 2.5–5% v/v lysed horse blood. For *Haemophilus influenzae*, plates and inocula were prepared in *Haemophilus* test medium (Trek Diagnostic Systems Ltd., East Grinstead, UK). For anaerobic isolates, supplemented *Brucella* broth was prepared according to CLSI recommendations [13,14].

2.5. Determination of bactericidal activity by time–kill

XF-73, vancomycin, fusidic acid and mupirocin were added at final concentrations of 2×, 4× and 8× MIC to exponentially dividing cultures of the test strains. A higher final bacterial concentration of ca. 10^6 CFU/mL was utilised to allow a ca. $5\log_{10}$ reduction to be measured. Cultures were incubated at $37 \pm 1\,^{\circ}C$ in a shaking water-bath and samples were removed for determination of surviving bacteria using the spiral plating system (Don Whitley Scientific, Shipley, UK) on Iso-Sensitest agar after 0, 0.25, 0.5, 1, 3, 6 and 8 h of exposure. To remove the antimicrobials prior to performing viable counts, each time point sample was spun and washed twice with sterile Iso-Sensitest broth. A growth control was run under the same conditions. Count plates were incubated for 48 h and the kinetics of bactericidal activity was determined by plotting the concentration of surviving bacteria against time. The absolute limit of detection was 2×10^1 CFU/mL.

2.6. Determination of the post-antibiotic effect

The PAE is the duration of bacterial growth suppression following abrupt removal of the test antibacterial from the culture medium [16–18]. This duration is related to the antibacterial concentration and exposure time prior to its removal [16] and is also specific for the antibacterial–bacterium system [16,18].

XF-73 was added at a final concentration of 0.5×, 1× and 2× MIC and vancomycin at a final concentration of 2× MIC to exponentially dividing cultures of the test strains in Iso-Sensitest broth (final concentration ca. 10^6 CFU/mL). Following exposure for 30 min, each culture was spun and washed twice with sterile Iso-Sensitest broth and the re-suspended culture was then incubated in a shaking water-bath at $37 \pm 1\,^{\circ}C$ until re-growth was visible with the naked eye or for a period of 8 h. The control culture was diluted 1:100 and 1:1000 to account for antibacterial activity that may occur in the exposed cultures in order to provide a comparable starting inoculum on re-incubation for PAE calculation. The three controls were also spun and washed before sampling all of the cultures, at time point 0. Thereafter, both control and 'exposed' cultures were counted at hourly intervals using the spiral plating method.

Count plates were incubated for 48 h and the PAE was determined by plotting the concentration of surviving bacteria against time. The PAE was calculated as the time taken for the test culture to increase by $1.0\log_{10}$ CFU/mL minus the time taken for the control culture to increase by $1.0\log_{10}$ CFU/mL.