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Spectrum of activity, mutation rates, synergistic interactions, and the effects of pH and serum proteins for fusidic acid (CEM-102)

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Abstract

Fusidic acid (CEM-102) is a steroidal antimicrobial agent with focused Gram-positive activity that acts by preventing bacterial protein synthesis via interacting with elongation factor G. A collection of 114 wild-type isolates (>80 species) was used to define the contemporary limits of fusidic acid spectrum against Gram-positive and Gram-negative species. Reference broth microdilution and anaerobic agar dilution methods were performed. Modifications of standardized test methods included adding 10% human serum and adjusting the medium pH to 5, 6, and 8. Synergy was assessed by the checkerboard method and time-kill studies. Mutational rates to resistance were determined at 4×, 8×, and 16× MIC. Against Gram-positive pathogens, fusidic acid MIC values ranged from 0.06 to 32 μg/mL with the greatest potency against *Staphylococcus aureus*, *Corynebacterium* spp., and *Micrococcus luteus* (MIC results, 0.25, ≤0.12, and ≤0.5 μg/mL, respectively). Enterococci and streptococci were less susceptible (MIC ranges, 2–8 and 16–32 μg/mL, respectively). Fusidic acid activity against Gram-negative species was more limited (all MIC values, ≥2 μg/mL) except for *Empedobacter brevis*, *Moraxella catarrhalis* and *Neisseria meningitidis*. A 4-fold increase in fusidic acid MIC results was observed when 10% serum was added to the broth. Decreasing medium pH to 5.0 to 6.0 negated the protein binding effects. Among the 8 antimicrobial combinations tested, gentamicin and rifampin enhanced the activity when combined with fusidic acid (no antagonism). Fusidic acid in vitro activity was most improved when combined with rifampin. Single-step mutational rates ranged from 1.2×10^{-6} for 4× MIC to 9.8×10^{-8} for 16× MIC. In conclusion, these in vitro results for fusidic acid tested against contemporary strains confirm a persisting antimicrobial spectrum, especially against staphylococci and some other Gram-positive species.

Keywords: Fusidic acid; CEM-102; Gram-positive activity; *S. aureus*; Synergy

1. Introduction

Overall, *Staphylococcus aureus* is the most common pathogen associated with serious Gram-positive bacterial infections, and this species is currently one of the leading threats to public health worldwide with regard to morbidity, mortality, and health-care costs (Corey, 2009; Lode, 2009). The prevalence of methicillin (oxacillin)-resistant *S. aureus* (MRSA) has increased over the past several decades in most countries, and both community-associated (CA) and hospital-acquired strains can be resistant to multiple antimicrobial classes or have significant virulence factors (Cornaglia and Rossolini, 2009; Lode, 2009; Stryjewski and Chambers, 2008). Beginning in the 1980s, glycopeptides such as vancomycin and teicoplanin were determined to be the only treatment options available for infections caused by MRSA strains that are also multidrug resistant (MDR). This paradigm is now under debate after the isolation of glycopeptide-resistant *S. aureus*, currently isolated only in the United States, and the more common detection of vancomycin-intermediate or heteroresistant strains (Deresinski, 2009; Howden et al., 2004).

The Clinical and Laboratory Standards Institute (CLSI) changed the susceptibility breakpoint criteria for vancomycin in 2006 to enhance the detection of *S. aureus* isolates that may not respond to vancomycin therapy (Tenover and Moellering, 2007). Increasing reports of vancomycin-

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nonsusceptible or tolerant strains of staphylococci and treatment failures using this agent has shifted the standard of care to more recently introduced antimicrobial agents such as linezolid and daptomycin (Deresinski, 2007; Hidayat et al., 2006; Howden et al., 2004). Unfortunately, resistance to these antimicrobial agents have now begun to emerge, and treatment failures have also been documented (Kainer et al., 2007; Lewis et al., 2005; Mendes et al., 2008; Skiest, 2006; Toh et al., 2007). In this era, having an increasing prevalence of MDR pathogens with limited treatment options, there are many advocates that support the introduction of older antimicrobial agents in countries or regions that have not yet experienced selective antimicrobial resistance pressure (Anderson, 1980; Howden and Grayson, 2006).

CEM-102 (fusidic acid) is a steroidal antimicrobial agent that was characterized in the 1960s from culture filtrates of *Fusidium coccineum* and was first introduced into clinical practice in 1962 (Anderson, 1980). Fusidic acid has potent antimicrobial activity against some Gram-positive pathogens including MRSA. This agent has been administered by multiple routes including oral, intravenous, and topical applications and can be used for systemic treatment of cutaneous infections or osteomyelitis. Topical applications can be used for primary and secondary skin infections, and ophthalmic solutions are available for superficial infections of the eye and conjunctiva. Fusidic acid has been used for over 4 decades in many countries worldwide and was introduced into Canada in the 1980s but has not yet been approved for use in the United States (Anderson, 1980). Fusidic acid acts by interfering with elongation factor G (EF-G), which inhibits polypeptide chain elongation. It remains equivocal if this mode of action results in bacteriostatic or bactericidal activity, which may also be dependent upon the inoculum concentration or the targeted bacterial species. Resistance to fusidic acid can occur as spontaneous mutations in the EF-G or by acquired resistance mechanisms (Lannergard et al., 2009). However, even after decades of fusidic acid use, resistance rates have remained low in most countries, although remarkable differences in rates of resistance and occurrences/types of resistance mechanisms among *S. aureus* have been observed (Larsen et al., 2008; Laurent et al., 2009; Mason et al., 2003; McLaws et al., 2008; O'Neill et al., 2007; Rennie, 2006; Tveten et al., 2002).

With the potential introduction of fusidic acid into the United States after the completion of ongoing clinical trials, this study was conducted to evaluate multiple objectives. Although fusidic acid is active against *S. aureus*, other Gram-positive species, such as *Staphylococcus saprophyticus*, are intrinsically resistant to this agent (Collignon and Turnidge, 1999). One of the objectives for this study was to test a broad sample of Gram-positive and Gram-negative species to clearly define the limits of fusidic acid spectrum and potency. It has been determined that there has been a low genetic barrier to resistance to fusidic acid in *S. aureus*, with only a single point mutation required to produce resistance (Anderson, 1980; Lannergard et al., 2009). To define resistance selection, this study also evaluated the in vitro mutational frequency of fusidic acid in CA-MRSA isolates after a single exposure to this agent. It has also been observed that resistance is readily acquired when fusidic acid is used alone during the course of treatment, but not when used in combination with other antimicrobial agents (Anderson, 1980; Howden and Grayson, 2006). To document this further, this study tested potential interactions (synergism to antagonism) of fusidic acid when combined with other agents tested against *S. aureus* isolates. Fusidic acid is a weak acid with a p*K* of 5.3 and also has significant protein binding when tested by reference methods at a medium pH of 7.2 to 7.4. However, less is known about the effects of pH variation (infection environments, such as abscesses) and proteins on fusidic acid potency. Therefore, the final objective of this study was to determine the effects of pH and serum proteins on the activity of this older agent.

2. Materials and methods

2.1. Bacterial strains

Fusidic acid and comparator agents were susceptibility tested against 114 Gram-positive and Gram-negative organisms, including quality control (QC) reference strains. These bacterial isolates included 27 species of Gram-positive aerobes: *S. aureus*, *Micrococcus luteus*, *Enterococcus* spp. (5 species) (Table 1), *Streptococcus agalactiae*, viridians group streptococci (12 species) (Table 1), *Bacillus cereus*, *Corynebacterium jeikeium*, *Corynebacterium xerosis*, *Leuconostoc* spp., *Listeria monocytogenes*, *Rothia mucilaginosa*, and *Weissella confusa*. A total of 20 species of Enterobacteriaceae from 13 genus groups and 20 species from 17 genus groups of Gram-negative nonfermentative bacilli were also tested (Table 1). Fastidious pathogens included *Neisseria meningitidis* (2 strains), *Moraxella catarrhalis* (2 strains), and 1 strain each of *Haemophilus haemolyticus* and *Haemophilus parainfluenzae*. Gram-positive anaerobic pathogens included *Clostridium* spp. (5 species, 21 strains), *Peptostreptococcus* spp. (8 strains), and *Eggerthella lenta* (1 strain) as shown in Table 1. Five isolates of Gram-negative anaerobic pathogens including *Bacteroides fragilis* (2 strains) and *Bacteroides thetaiotaomicron* (3 strains) were also tested.

2.2. Antimicrobial susceptibility testing

The CLSI broth microdilution (frozen-form panels) and agar dilution methods were used for aerobic (CLSI, 2009a) and anaerobic organisms (CLSI, 2008), respectively. Cation-adjusted Mueller–Hinton broth (MHB) was used for testing the nonfastidious Gram-positive and Gram-negative isolates as well as *M. catarrhalis*. MHB was supplemented with 2% to 5% lysed horse blood for testing streptococci, *N. meningitidis*, and *Corynebacterium* spp. *Haemophilus*