203

Update on the Spectrum of CEM-102 (Fusidic Acid) Against Contemporary Wildtype Bacterial Species Including Mutational Resistance Analysis, and Synergy Testing

PR RHOMBERG, DJ BIEDENBACH, RE MENDES, HK BECKER, KA FEDLER, HS SADER, RN JONES

JMI Laboratories, North Liberty, Iowa, USA

IDSA 2009

JMI Laboratories
North Liberty, IA, USA
www.jmilabs.com
319.665.3370, 319.665.3371
ronald-jones@jmilabs.com

Abstract

Background: Fusidic acid (FA) is a steroidal antimicrobial agent with focused Gram-positive activity (skin and skin structure infections) which acts by preventing bacterial protein synthesis via interacting with elongation factor G.

Methods: A collection of 114 wild type (WT) isolates (>80 species) was used to define the contemporary limits of FA spectrum against Gram-positive (GP) and -negative (GN) species. CLSI broth microdilution (BMD) and anaerobic agar dilution (AD) methods were performed. Modifications of standard test methods included adding 10% human serum, adjusting the medium pH to 5, 6, and 8 and synergy was assessed by the checkerboard method. Mutational rates to resistance (R) were determined at 4x, 8x and 16x MIC.

Results: Against GP FA MIC values ranged from 0.06-32 µg/ml with greatest potency against *S. aureus*, *Corynebacterium* spp. and *M. luteus* (MIC results 0.25, ≤0.12 and ≤0.5 µg/ml, respectively). Enterococci and streptococci were less susceptible (S; MIC ranges of 2-8 and 16-32 µg/ml, respectively). FA activity against GN species was limited (all MIC values ≥2 µg/ml) except for *E. brevis*, *M. catarrhalis* and *N. meningitidis* (MICs, 0.12-0.5 µg/ml). A 4-fold increase in FA MIC results was observed when 10% serum was added. Decreasing medium pH to 5.0-6.0 negated the protein binding effects. Among the 8 combinations tested, gentamicin (GEN) and rifampin (RIF) showed the greatest enhanced activity combined with FA (No antagonism). Single-step mutational rates ranged from 1.2x10^{-6} for 4x MIC to 9.8x10^{-8} for 16x MIC.

	Synergy					
FA/co-drug	Complete	Partial	Additive	Indifferent	Antagonism	Indeterminate
Rifampin	0	5	1	0	0	0
Levofloxacin	0	0	0	4	0	2
Gentamicin	1	1	3	1	0	0
Oxacillin	0	1	1	3	0	1
Vancomycin	0	0	2	4	0	0
All agents tested	1	7	9	24	0	7

Conclusions: FA demonstrated potent GP activity, especially against the staphylococci. A more limited activity was observed against GN species isolates. Added serum proteins adversely influenced MIC values; however lower media pH, like seen at infection sites, decreased negative protein binding effects. FA in vitro activity was most improved when combined with RIF.

Introduction

Staphylococcus aureus is currently one of the leading threats to public health worldwide with regards to morbidity, mortality and healthcare costs. Isolation of vancomycin-resistant *S. aureus* (VRSA), -intermediate (VISA) or hetero-resistant (hVISA) strains and increasing reports of treatment failures have shifted the standard of care more toward linezolid and daptomycin. Unfortunately, resistances to these antimicrobial agents have also emerged. There are many advocates that support the use of older agents in countries that have not yet experienced selective antimicrobial pressure for those drugs.

CEM-102 (fusidic acid) is a steroidal antimicrobial agent, introduced into clinical practice in 1962. Fusidic acid has potent antimicrobial activity against Gram-positive pathogens including MRSA and has been used for over four decades in many countries, but has not yet been approved for use in the United States (USA). Even after decades of fusidic acid use, resistance rates have remained low in most countries.

This study was conducted to evaluate multiple objectives, including testing a broad sample of Gram-positive and Gram-negative species to clearly update and define the limits of fusidic acid spectrum and potency. We also defined resistance selection after a single exposure to this agent, the potential interactions (synergism to antagonism) with other agents and determined the effects of pH and serum proteins on the activity of this agent.

Materials and Methods

A total of 114 Gram-positive and -negative organisms were susceptibility tested against fusidic acid to determine the spectrum of activity. These bacterial isolates included 27 species of Gram-positive aerobes, 20 species of Enterobacteriaceae from 13 genus groups and 20 species from 17 genus groups of Gram-negative non-fermentative bacilli. Fastidious pathogens included *Neisseria* spp., *Moraxella catarrhalis*, *Haemophilus* spp. and the anaerobes *Clostridium* spp., *Peptostreptococcus* spp. *Eggerthella lenta*, *Bacteroides fragilis* and *B. thetaiotaomicron*.

The Clinical and Laboratory Standards Institute (CLSI) broth microdilution methods in frozen-form panels and agar dilution methods were used for aerobic (M07-A8, 2009) and anaerobic organisms (M11-A7, 2007), respectively. Appropriate CLSI media, supplements and incubation environments were used in order to support the growth of challenge species. Quality control (QC) ranges and interpretive criteria for comparator compounds were as published in the CLSI M100-S19 (2009) using appropriate ATCC strains. All QC results were within published limits.

To determine single-step mutation rates, isolates were suspended in sterile MHB to achieve approximately 1 to 5 x 10^{9} colony forming units (CFU)/ml. Inoculum suspensions were plated onto agar plates containing 4x, 8x and 16x MIC of fusidic acid. Colony counts of the mutant population were determined after 24 and 48 hours of incubation and the mutation frequency was determined as the ratio of mutants divided by the total number of bacteria in the initial population.

Broth microdilution checkerboard trays (MHB) were used to determine the potential synergistic activity between fusidic acid and antimicrobial agents from other classes. Confirmatory kill curve studies with fusidic acid and co-drugs rifampin and gentamicin, which showed the greatest degree of synergy, were determined alone at one-half and 2x the MIC and co-drugs were tested at one-fourth the MIC alone and in combination with the two fusidic acid concentrations.

The effects of added serum proteins and media pH levels on the activity of fusidic acid was determined using various pH and protein contents, including four different pH values (5.0, 6.0, 7.2-7.4 [physiological level], and 8.0) and 10% pooled serum protein which was added and compared to results obtained with MHB free of supplemental proteins.

Results

- The fusidic acid MIC values for Gram-positive aerobic organisms (Table 1) ranged from 0.06 µg/ml to >32 µg/ml, with the lowest values for *S. aureus* (0.25 µg/ml), *Corynebacterium* spp. (≤0.12 µg/ml) and *Micrococcus luteus* (≤0.5 µg/ml).
- Modest fusidic acid activity was observed against enterococci, streptococci and other infrequently isolated Gram-positive pathogens with MIC values of 2 to >32 µg/ml (Table 1). This compound did not exhibit activity against Enterobacteriaceae (MIC values, >32 µg/ml) and most Gram-negative non-fermentative bacilli (Table 1).
- *M. catarrhalis*, *N. gonorrhoeae* and *N. meningitidis* had MIC values that ranged from 0.06 to 2 µg/ml and *Haemophilus* spp. had MIC values at ≥8 µg/ml.
- Fusidic acid was more active against Gram-positive anaerobes with MIC values ranging from 0.5 to 8 µg/ml compared to Gram-negative species with MIC values 8 to 16 µg/ml (Table 1).
- Table 2 shows that at 4x and 8x the fusidic acid MIC, the frequencies of mutants ranged from 1.2 x 10^{-6} to 2.9 x 10^{-7}, but at 16x the MIC, the mutational rate was only 7.4 to 9.8 x 10^{-8}.
- The dominant interaction category (24/41 occurrences; 59%) for fusidic acid synergy studies was “indifference”. This was followed by “additive” interactions at 9/41 occurrences or 22%. No antagonistic combinations were observed (Table 3).
- All six organisms showed “partial synergy or additive” interactions for the fusidic acid/rifampin combination, and 5 of 6 organisms with the fusidic acid/gentamicin combination showed improved activity (≥ additive; Table 3). Figure 1a shows enhanced killing of the fusidic acid/rifampin combination compared to the best single agent tested against *S. aureus* ATCC 25923, confirming observations from the checkerboard synergy studies. Results at 8 and 24 hours of incubation show slightly enhanced killing for the combination of fusidic acid/gentamicin compared to gentamicin alone for *S. aureus* ATCC 29213 (Figure 1b).
- As the media pH increases, the fusidic acid MIC value increases, decreasing potency (Table 4). Similarly, adverse effects of 10% human serum protein on fusidic acid activity was demonstrated by a four-fold increase in the MIC using the reference broth microdilution testing conditions (pH 7.2-7.4 ± 10% human serum proteins).

Table 1. Spectrum of antimicrobial activity of fusidic acid tested against Gram-positive and –negative aerobic and anaerobic bacterial pathogens.

Organism group/species (no. tested)	MIC range (µg/ml)
Gram-positive aerobes (29)	
S. aureus (1)	0.25
M. luteus (2)	0.25-0.5
Enterococcus spp. (6)[a]	2-8
Streptococcus spp. (13)[b]	16-32
R. mucilaginosa (1)	8
W. confusa (1)	32
Leuconostoc spp. (1)	>32
Corynebacteria spp. (2)	0.06-0.12
B. cereus (1)	4
L. monocytogenes (1)	16
Gram-positive anaerobes (30)[c]	
Clostridium spp. (21)	0.5-8
Peptostreptococcus spp. (8)	1-4
E. lenta (1)	8
Gram-negative Enterobacteriaceae (21)[d]	>32
Gram-negative non-fermentative bacilli (23)[e]	0.5->32
Gram-negative anaerobes (5)	8-16
Fastidious species (41)	
Haemophilus spp. (2)	8-32
M. catarrhalis (2)	0.06-0.12
Neisseria spp. (37)[f]	0.12-2

a. Included: *E. avium, E. casseliflavus, E faecium, E gallinarum* and *E. faecalis* (2 strains).
b. Included: *S. agalactiae, S. anginosus, S. bovis, S. constellatus, S., gordonii, S. intermedius, S. milleri, S. mutans, S. oralis, S. parasanguinis, S. salivarius, S. sanguine* and *S. uberis*.
c. Included: *C. clostridioforme, C. terium, C. butyricum* (2 strains), *C. difficile* (13 strains), *C. perfringens* (4 strains), *P. asaccharolyticus* (2 strains), *P. micros* (3 strains), and unspeciated *Peptostreptococcus* spp. (3 strains)
d. Included: *Citrobacter freundii, C. koseri, Enterobacter aerogenes, E. cloacae, Hafnia alvei, Leclercia adecarboxylata, Klebsiella oxytoca, K. pneumoniae, Kluyvera* spp., *Morganella morganii, Pantoea agglomerans, Proteus mirabilis, P. vulgaris, Providencia rettgeri, P. stuartii, Salmonella* group C., *Salmonella* group D, *Serratia liquefaciens, S. marcescens* and *Yersinia enterocolitica* (2 strains).
e. Included: *Achromobacter xylosoxidans, Acinetobacter baumannii, A. junii, A. lwoffi* (3 strains), *Aeromonas sobria, Agrobacterium radiobacter, Burkholderia cepacia, Bordetella bronchiseptica* (2 strains), *Chryseobacterium indologenes, Delftia acidovorans, Empedobacter brevis, Kingella kingae, Ochrobactrum anthropi, Pasteurella multocida, Pseudomonas putida, P. stutzeri, Ralstonia pickettii, Shewanella algae, Sphingomonas paucimobilis* and *Stenotrophomonas maltophilia*
f. Included: 35 isolates of *N. gonorrhoeae* and two isolates of *N. meningitidis*.

Table 2. Frequency of mutation with a single exposure to fusidic acid when testing two methicillin-resistant *S. aureus* isolates (CA-MRSA; USA300).

Organism	Fusidic acid concentration	Frequency of mutation[a]
011-966D	4x MIC	1.2 x 10^{-6}
	8x MIC	2.9 x 10^{-7}
	16x MIC	9.8 x 10^{-8}
044-59D	4x MIC	2.7 x 10^{-7}
	8x MIC	1.3 x 10^{-7}
	16x MIC	7.4 x 10^{-8}

a. Initial inoculums were 3.8 to 4.0 x 10^{9} CFU/ml.

Table 3. Fusidic acid drug interaction (synergy) categories tested in combination with eight other antimicrobials.

	Occurrences by interaction category:					
	Synergy					
Fusidic acid co-drug	Complete	Partial	Additive	Indifferent	Antagonism	Indeterminate
Rifampin	0	5	1	0	0	0
Ciprofloxacin	0	0	0	4	0	2
Levofloxacin	0	0	0	4	0	2
Gentamicin	1	1	3	1	0	0
Oxacillin	0	1	1	3	0	1
Ceftriaxone	0	0	2	2	0	2
Vancomycin	0	0	2	4	0	0
Aztreonam[a]	-	-	-	6	-	-
All	1	7	9	24	0	7

a. Aztreonam tested at a single susceptible breakpoint concentration (8 µg/ml), only. Five of six strains showed no change in the fusidic acid MIC and one strain had an increase from 0.06 to 0.12 µg/ml (susceptible).

Figure 1. Kill curve results for fusidic acid (CEM-102) tested in combination with rifampin against *S. aureus* ATCC 25923(a) and gentamicin against *S. aureus* ATCC 29213(b).

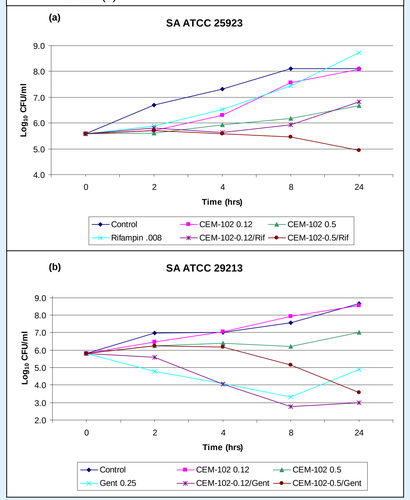

Table 4. In vitro influences of medium pH ± 10% human serum protein on fusidic acid activity tested against five *S. aureus* strains.

		MIC at pH (µg/ml):			
Organisms	10% serum	5.0	6.0	7.2-7.4	8.0
S. aureus 17-23A	-	0.25	0.25	0.25[a]	0.5
	+	0.12	0.25	1	2
S. aureus 27-41X	-	0.25	0.25	0.25[a]	0.5
	+	0.12	0.25	1	2
S. aureus 716J	-	0.25	0.25	0.25[a]	0.5
	+	0.12	0.25	1	2
S. aureus ATCC 29213	-	0.25	0.25	0.25[a]	1
	+	0.12	0.25	1	2
S. aureus ATCC 25923	-	0.25	0.25	0.25[a]	1
	+	0.12	0.25	1	2

a. Reference MIC at pH 7.2-7.4 without added human serum proteins.

Conclusions

- The spectrum of fusidic acid activity is limited to some Gram-positive aerobic pathogens, including staphylococci, micrococci and *Corynebacterium* spp. and selected fastidious Gram-negative species including, *N. meningitidis*, *N. gonorrhoeae* and *M. catarrhalis*.
- Results from this study suggest low mutational frequency and potential synergistic activity for fusidic acid with other antimicrobial classes when tested against *S. aureus* isolates.
- Fusidic acid is an older antimicrobial agent with continued potential clinical utility against some important bacterial pathogens, including MRSA, especially in the USA.

References

1. Anderson JD (1980). Fusidic acid: new opportunities with an old antibiotic. *Can Med Assoc J* 122: 765-769.
2. Clinical and Laboratory Standards Institute (2009). *M07-A8. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard - eighth edition.* Wayne, PA:CLSI.
3. Clinical and Laboratory Standards Institute (2009). *M100-S19. Performance standards for antimicrobial susceptibility testing. 19th informational supplement* Wayne, PA:CLSI.
4. Dobie D, Gray J (2004). Fusidic acid resistance in *Staphylococcus aureus*. *Arch Dis Child* 89: 74-77.
5. Howden BP, Grayson ML (2006). Dumb and dumber--the potential waste of a useful antistaphylococcal agent: emerging fusidic acid resistance in *Staphylococcus aureus*. *Clin Infect Dis* 42: 394-400.