Relevance of Protein Binding of CEM-102 (Fusidic acid) and its pH-dependent Effect on in vitro Activity

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Abstract

Background: The effect of drug concentration on protein binding and the pH-dependent effect of protein binding on the in vitro activity of CEM-102 (fusidic acid), an oral antibiotic in development for the treatment of acute bacterial skin structure infections, was examined.

Methods: Mouse and human plasma, human serum albumin (HSA, 50 mg/mL) and α1-acid glycoprotein (AAG, 1 mg/mL) were spiked with CEM-102 at 75, 750, and 7500 ng/mL and ultrafiltrates were analyzed for CEM-102 concentrations by LC/MS/MS. Susceptibility tests against *Staphylococcus aureus* were performed with and without 10% human serum at various pH ranges in Mueller Hinton (MH) broth, using a modification of standard CLSI methods.

Results: CEM-102 binds to mouse and human plasma with approximately the same degree of affinity (97.0-97.9%). In human blood, CEM-102 binds primarily to HSA (binding ratios 96.3-96.8%), but does not bind significantly to AAG (0.5-9.8%). Binding was not concentration dependent (75-7500 ng/mL), indicating that there is no saturation of binding sites at the concentrations studied. MIC values (total of 7 MSSA and MRSA) increased 4 to 8-fold in the presence of 10% serum at pH 7.2-7.4. At pH 6.0 the effect of serum was negated and the MICs were restored to 0.12 -0.25 µg/mL, identical to the MICs in MH broth without serum. This would be expected based on the low pKa of 5.7 of CEM-102, suggesting that the extent of protein binding to CEM-102 is less in acidic environments, such as those commonly encountered in sites of infection.

Conclusions: CEM-102 MICs for *S. aureus* (7 strains) increased 4 to 8-fold in the presence of serum. However, this effect was mitigated in a more acidic milieu, at pH ranges below 7.2. These results indicate that although CEM-102 binds highly to plasma protein, this binding appears to be weak, reversible, and pH dependent.

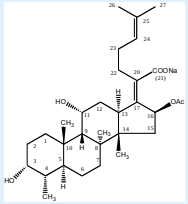
Introduction

CEM-102 (Figure 1) is sodium fusidate, an antibiotic of the fusidane class that is under development in the US by Cempra Pharmaceuticals for treatment of patients with acute bacterial skin structure infections. CEM-102 has shown excellent microbiological activity against *Staphylococcus aureus*, including methicillin resistant *S. aureus* (MRSA) (MIC_{90} 0.12 µg/mL). Coagulase-negative staphylococci (CoNS) with or without oxacillin-resistance were all highly susceptible to CEM-102 (MIC_{90} 0.12 µg/mL). The MIC range of CEM-102 against more than 100 isolates of Group A streptococci was 2 to 8 µg/mL.

Sodium fusidate is highly bound to plasma proteins. At plasma pH, sodium fusidate is largely ionized with a high intrinsic affinity to albumin, which under normal conditions can bind 2-3 molecules (Rieutord, 1995). Protein binding is generally reversible and because sodium fusidate is a weak acid with a pK_a of 5.35, a change in protein binding at lower pH ranges is conceivable. Good tissue distribution of sodium fusidate has been reported previously and it appears that protein binding of sodium fusidate does not prevent its penetration into interstitial fluid. The persistent high concentrations gradient provided by its long half-life (11-16 hours), its lipophilic properties, the local pH and binding to cellular components are important in producing high interstitial concentrations (Vaillant, 1992). The objectives of the studies described here were as follows:

1) Study the binding of CEM-102 to mouse and human plasma, including relevant individual plasma proteins, such as human serum albumin (HSA) and α1-acid glycoprotein (AAG)
2) Explore the effect of pH and protein binding on the in vitro activity against a variety of *S. aureus* strains

Figure 1. Chemical Structure of CEM-102



Study Design and Methodology

Protein Binding

- Separate solutions with nominal concentrations of 75, 750, and 7500 ng/mL of CEM-102 were prepared in mouse and human plasma, HSA (50 mg/mL) and AAG (1 mg/mL) using a 1 mg/mL stock solution of CEM-102 in water:acetonitrile (1:1)
- Ultrafiltration: A 1 mL aliquot of each spiked plasma sample was loaded into a Centrifree cartridge and centrifuged at 1300·g for 25 minutes. The prefiltered plasma and ultracentrifugate samples were collected and extracted.
- Extraction: Plasma samples (50 µL) and ultracentrifuged samples (100 uL) were extracted with 250 µL of extraction buffer (0.05 M citric acid, 0.2 M ammonium phosphate in water) and 3 mL of extraction solvent (dichloromethane: hexane:MTBE, 1:1:1). Samples were mixed and frozen, then the organic layer was transferred into injection vials for HPLC analysis.
- HPLC Analysis: Samples were injected onto a HPLC system using a Synergi Hydro RP column (Phenomenex). The gradient was isocratic with 16% solvent A (0.05% acetic acid in water) and 84% solvent B (0.05% acetic acid in acetonitrile:methanol, 70:30) at a flow rate of 0.3 mL/min. Samples containing CEM-102 and tetrahydrofusidic acid as internal standard were nebulized with heated nitrogen using a Z-spray source/interface and the ionized compounds were detected by quadrupole tandem mass spectrometry.
- Calculation of %Binding to Protein:

  % Protein Binding = $(C_B/C_T) * 100$

  C_T = Total concentration in plasma

  C_B = Bound concentration $(C_T - C_F)$

  C_F = Free concentration in ultrafiltrate

MIC determinations

- Representative staphylococcal isolates were tested against CEM-102 in Mueller-Hinton broth (MHB) at various pH values with or without added serum protein
- Clinical and Laboratory Standards Institute (CLSI) broth microdilution method was used
- MHB was supplemented for:
  - 4 different pH conditions (5.0, 6.0, 7.2-7.4, and 8.0)
  - 10% serum protein was added and results compared to MHB free of supplemented proteins
- 5 *S. aureus* strains were assessed
  - 3 wild-type MRSA strains
  - 2 QC MSSA strains

Results

Protein Binding

- No significant binding of CEM-102 to the ultrafiltration device was observed
- CEM-102 was significantly bound to mouse and human plasma and HSA. Very low binding was observed to AAG (Table 1)
- Binding was not concentration dependent for CEM-102 concentrations of 75-7500 ng/mL

Table 1. Protein Binding of CEM-102

Matrix	Target Concentration (ng/mL)	Concentration in Protein (ng/mL)	Concentration in Filtrate (ng/mL)	%Bound
Mouse Plasma	75	75.9	1.73	97.7
	750	800	17.1	97.9
	7500	7217	217	97.0
Human Plasma	75	72.2	1.66	97.7
	750	767	19.3	97.5
	7500	7550	209	97.2
HSA	75	77.3	2.49	96.8
	750	798	26.0	96.7
	7500	7497	278	96.3
AAG	75	48.4	47.8	1.24
	750	472	426	9.75
	7500	5850	5820	0.513

Effect of pH and protein binding on MICs against *S. aureus* strains

- As the pH increases, the MICs of CEM-102 increase. They were greatest at pH 8.0 with added human serum proteins in the medium (Table 2).
- Human serum protein on CEM-102 activity was demonstrated by a 4-fold (average) increase in MIC using reference broth microdilution testing conditions (pH 7.2-7.4 ± 10% human serum proteins)
- The effect of protein binding on CEM-102 activity observed at pH 7.2-8.0 was negated by testing conditions approaching the CEM-102 pK of 5.35 (pH 5.0-6.0).
- Differences in CEM-102 MIC values (usually 0.12 or 0.25 µg/mL) in the presence or absence of human serum protein were minimal when tested in media having a pH of 5.0-6.0.
- The 4-fold increase in MIC was less than would be predicted for an antibiotic bound >96% by human serum protein. This low correlation between high protein binding and small change in MIC may be related to the weak ionic binding of CEM-102 to albumin (Rieutord, 1995)

Table 2. Influence of medium pH ± 10% human serum protein on CEM-102 in vitro activity against 5 *S. aureus* strains.

Organisms	10% serum	MIC at pH (µg/mL) 5.0	6.0	7.2-7.4	8.0
MRSA					
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
MSSA					
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

- CEM-102 is significantly bound to mouse and human plasma proteins.
- Human serum albumin is the primary protein involved in binding CEM-102 as CEM-102 binding to AAG is very low. The binding to serum albumin has been shown previously to be a weak and reversible ionic interaction (Rieutord, 1995).
- The potential adverse influences of protein binding on CEM-102 activity are markedly reduced in acidic (pH <7.2-7.4) testing environments such as those encountered at the site of bacterial infections.
- CEM-102 MICs against all MRSA and MSSA strains did not exceed 2 µg/mL even at pH 8.0 in the presence of serum. This increase in MIC is less than would be expected based on high protein binding, and may be related to the weak and potentially reversible binding of CEM-102 to albumin.

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