Contrasting Effect of Acidic pH on the Batericidal Activities of CEM-102 (Fusidic Acid) vs. Linezolid and Clindamycin Towards *Staphylococcus aureus*

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ABSTRACT

Background. *S. aureus* shows high tolerance to acidic pH. Acidity, however, may affect antibiotic activity. We have compared the influence of acidic pH on the activity of CEM-102 (a steroid-like antibiotic carrying a free carboxyl function) *vs.* LNZ and CLI against *S. aureus*.

Methods. *S. aureus* ATCC 25923 was grown in Mueller-Hinton broth (MHB). MICs were determined in MHB adjusted to pH 7.4 or pH 5.5. Dose-effect relationships at 24 h were examined for concentrations from 0.01 to 100 x the MIC. Results, expressed as the change in the inoculum at 24 h compared to time 0 h (T_0), were used to fit a Hill equation to allow determination of the values of two key pharmacological descriptors of antibiotic activity (relative potency [EC_{50} or 50% effective concentration] and maximal relative efficacy [E_{max}]; see Barcia-Macay et al, AAC 50(3):841-51).

Results. All dose-effect relationships could be modeled using a sigmoidal function (Hill equation; R^2, >0.950). MICs and pharmacological descriptors at both pH values are shown in the Table.

	pH 7.4				pH 5.5			
	MIC	E_0 [a]	EC_{50} [b]	E_{max} [c]	MIC	E_0 [a]	EC_{50} [b]	E_{max} [c]
CEM-102	0.5	2.6 ± 0.2 A;a	2.0 ± 1.3 A;a	-1.9 ± 0.2 A;a	0.005	2.5 ± 0.2 A;a	0.3 ± 1.4 A;b	-1.6 ± 0.2 A;a
LNZ	2	2.4 ± 0.2 A;a	3.0 ± 1.4 A;a	-1.7 ± 0.3 A;a	2	2.3 ± 0.1 A;a	3.7 ± 1.3 B;a	-1.9 ± 0.2 A;a
CLI	0.125	2.2 ± 0.3 A;a	0.4 ± 1.5 A;a	-3.0 ± 0.4 B;a	1-2	2.2 ± 0.1 A;a	4.9 ± 1.5 B;b	-2.8 ± 0.2 B;a

[a] increase in log CFU compared to T_0 for an infinitely low concentration in antibiotic (bact. growth)
[b] concentration (mg/L) causing a reduction of the inoculum halfway between E_0 and E_{max}, as obtained by graphical intrapolation using the corresponding Hill equation
[c] decrease in log CFU compared to T_0 for an infinitely high concentration in antibiotic (bact. killing)

Statistical analysis: figures with different letters are significantly different from each other ($p < 0.05$)
- upper case letters: analysis per column (one-way ANOVA with Tukey test for multiple comparisons between each parameter for all drugs);
- lower case letters: analysis per row (unpaired, two-tailed t-test between corresponding parameters of extracellular and intracellular activities)

Acidic pH did not affect bacterial growth (in the absence of antibiotic [E_0]) but (i) markedly decreased the MIC and the EC_{50} of CEM-102; (ii) had no effect on linezolid; (iii) increased the MIC and EC_{50} of clindamycin. Maximal relative efficacies (E_{max}) remained unchanged, with absolute values for CEM-102 similar to those of linezolid but lower than those of clindamycin.

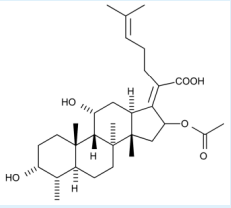
Conclusions. While maximal achievable efficacy is not modified, CEM-102 shows increased potency at acidic pH. This may confer an advantage to this molecule for infections localized in low pH environments, such as skin, urine, vagina, or phagolysosomes of infected cells

BACKGROUND AND AIM

Staphylococcus aureus is a widespread pathogenic bacterium showing high tolerance to pH variations. This confers an advantage for survival and colonization of body sites (see [1] for review) characterized by mild acidic pH, such as skin, vagina, urinary tract, or intracellularly, within the phagolysosomes of infected cells (pH ~ 5.0-5.5).

Acidity, however, may significantly decrease the activity of many antibiotics, as commonly observed for gentamicin (2) or azithromycin (3,4).

In this context, we have compared the influence of acidic pH on the activity of CEM-102 (fusidic acid [a steroid-like antibiotic carrying a free carboxyl function]) *vs*. linezolid (LNZ) and clindamycin (CLI) towards *S. aureus*.



Structure of CEM-102

METHODS

Bacterial strain: *S. aureus* ATCC 25923 (methicillin-sensitive) was used for all studies.

Susceptibility testing: MICs were determined in Mueller Hinton broth (MHB) adjusted to pH 7.4 or pH 5.5.

Killing curves in broth: Dose-effect relationships were examined at 24 h for increasing concentrations of antibiotic (0.01- to 100-fold the MIC). Results were expressed as the change in the inoculum at 24 h compared to T_0. Data were analyzed by non-linear regression using Hill's equation to calculate pharmacological descriptors (E_{max}, maximal reduction of the intracellular inoculum [in $\log_{10}$ units] for an infinitely large antibiotic concentration; E_0, increase in intracellular inoculum [in $\log_{10}$ units] for an infinitely low antibiotic concentration; EC_{50}, antibiotic concentration [in mg/L] yielding a response half-way between E_0 and E_{max}, as obtained by graphical intrapolation using the corresponding Hill equation).

REFERENCES

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(2) Baudoux et al, J Antimicrob Chemother (2007), 59:246-53.
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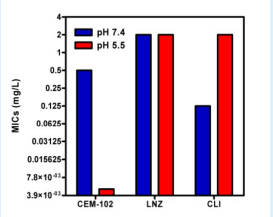
(4) Milatovic D., Eur J Clin Microbiol Infect Dis (1990), 9:33-5

RESULTS

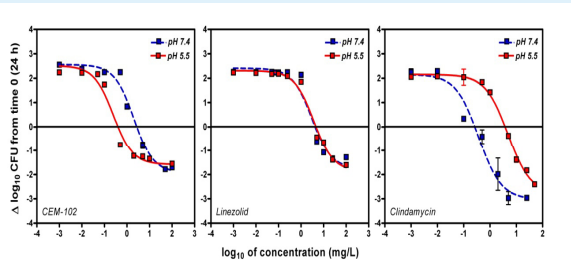
Influence of pH on the MICs of CEM-102, linezolid, and clindamycin



Against *S. aureus*, acidity :

- decreases the MIC of CEM-102
- does not affect the MIC of linezolid
- increases the MIC of clindamycin

Influence of pH on the activity of CEM-102, linezolid and clindamycin against *S. aureus*.



Concentration killing effects of CEM-102, linezolid and clindamycin against S. aureus strain ATCC 25923 in broth (pH 7.4 or pH 5.5). All values are means ± standard deviations.

Pharmacological descriptors (with statistical analysis) of the activity of antibiotics towards S. aureus at neutral and acidic pH (dose-response studies)

	pH 7.4			pH 5.5		
	E_0 [a]	EC_{50} [b]	E_{max} [c]	E_0 [a]	EC_{50} [b]	E_{max} [c]
CEM-102	2.6 ± 0.2 A;a	2.0 ± 1.3 A;a	-1.9 ± 0.2 A;a	2.5 ± 0.2 A;a	0.3 ± 1.4 A;b	-1.6 ± 0.2 A;a
LNZ	2.4 ± 0.2 A;a	3.0 ± 1.4 A;a	-1.7 ± 0.3 A;a	2.3 ± 0.1 A;a	3.7 ± 1.3 B;a	-1.9 ± 0.2 A;a
CLI	2.2 ± 0.3 A;a	0.4 ± 1.5 A;a	-3.0 ± 0.4 B;a	2.2 ± 0.1 A;a	4.9 ± 1.5 B;b	-2.8 ± 0.2 B;a

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Statistical analysis: figures with different letters are significantly different from each other ($p < 0.05$)
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- **Acidity did not affect bacterial growth (no change of E_0 values)**
- **Acidity exerted divergent effects on drug relative potencies (EC_{50} values) with an increase in potency (decreased EC_{50}) for CEM-102, no effect for linezolid, and a decrease in potency (increased EC_{50}) for clindamycin**
- **The maximal relative efficacies (E_{max}) for each drug remained unchanged**

CONCLUSIONS

In contrast with clindamycin and linezolid, we demonstrate that CEM-102 shows an increased relative potency at acidic pH. This may confer an advantage to this molecule for infections localized in mildly acidic compartments, such as the skin, the urine, the vagina, or the phagolysosomes of infected cells.

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