CEM-101, a Novel Macrolide/Ketolide, Shows Improved Intracellular Activity against Phagocytised *S. aureus* in Comparison with Azithromycin (AZM), Clarithromycin (CLR), or Telithromycin (TEL)

Poster A-995

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ABSTRACT

Background: Intracellular activity of antibiotics is an important determinant for fast and complete eradication and, probably also, for prevention of resistance. We have determined the intracellular activity of CEM-101, a novel macrolide/ketolide antibiotic, against *S. aureus* in comparison with azithromycin and clarithromycin (macrolides) and telithromycin (ketolide).
Methods: MICs and extracellular activities of antibiotics were determined in MHB at both neutral and acidic pH. Intracellular activity was determined against *S. aureus* (ATCC 25923) phagocytosed by THP-1 macrophages as previously described (AAC, 2006, 50:841-851). Results were expressed as a change of efficacy compared to time 0 h.
Results:

Cond.	AZI	CLR	TEL	CEM-101
MICs (mg/L)				
pH 7.4	0.5	0.5	0.5	0.125
pH 5.5	256	16	8	1-2
Broth pH 7.4				
Cs (mg/L)	~ 3.63	~ 1.41	~ 0.28	~ 0.06
Emax	-1.2 ±0.6	-1.4 ±0.2	-1.0 ±0.4	-1.4 ±0.1
Broth pH 5.5				
Cs (mg/L)	/	~ 10.47	~ 9.33	~ 1.48
Emax	2.1 ±0.1	-1.5 ±0.8	-1.4 ±0.9	-1.6 ±0.4
THP-1				
Cs (mg/L)	~ 10	~ 0.98	~ 0.28	~ 0.02
Emax	0.1 ±0.1	-0.1 ±0.1	-0.4 ±0.1	-0.8 ±0.2

[1] Maximal decrease of intracellular cfu compared to initial, post-phagocytosis inoculum (calculated from non-linear regression [sigmoidal] of dose-effect response) run in broth (extracell.) or with infected macrophages (intracell.)
[2] Extracellular concentration (in mg/L) yielding an apparent static effect

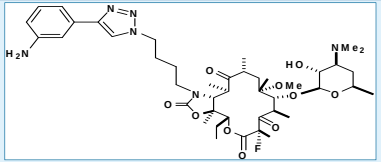
Conclusions:
Compared to AZM, CLR and TEL, CEM-101 activity was less affected by acidic pH in broth and showed greater potency (lower static dose) and larger maximal efficacy (Emax) against intracellular S. aureus.

BACKGROUND AND AIM

- Survival of *S. aureus* within eukaryotic cells is critical for the persistence of infection.[1] However, the selection of appropriate agents remains challenging, since routine susceptibility testings are usually determined against extracellular bacterial only (misjudging, therefore, the importance of the intracellular environment in the modulation of the pharmacokinetic and pharmacodyn-amic properties of the corresponding antibiotics).

  For example, the acidic environment prevailing in the phagolysosomes (where *S. aureus* sojourns during its intracellular stage) may impair the activity of antibiotics, such as the macrolide azithromycin.[2-4]

- In the present study, we aimed at investigating the intracellular activity of the novel **macrolide/ketolide CEM-101.** As will be shown, this compound, indeed, remains active at acid pH, and,as shown in the companion abstract shows extended cellular accumulation by macrophages (see Poster A-996)



MATERIAL AND METHODS

- *S. aureus* ATCC 25923 (macrolide susceptible) was used through our experiments.
- MICs (microdilution method) and extracellular activities were determined in Mueller Hinton Broth as described previously ([3,5]).
- Intracellular activities were determined against *S. aureus* post- phagocytized by human THP-1 macrophages, as described previously([3,5]).

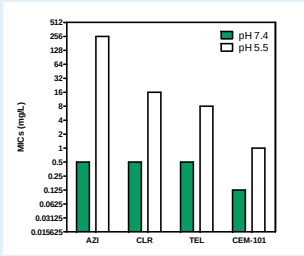
CONCLUSIONS

CEM-101 is a promising antistaphylococcal agent owing to its:

- Higher activity against extracellular *S. aureus*, even under acidic conditions (while azithromycin failed to prove activity)
- Larger activity against intracellular forms, compared to the comparators tested (AZI, CLR and TEL)

RESULTS

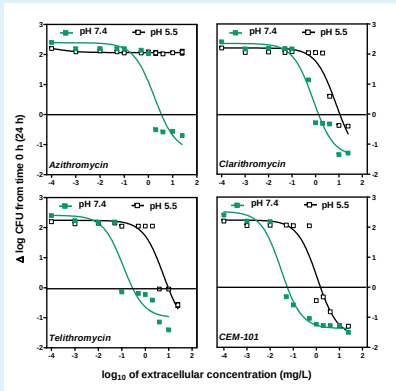
Susceptibility testing in broth against *S. aureus* ATCC 25923:



Comparative pharmacological descriptors (E_{max} and static concentrations [C_s]) obtained from the dose-responses studies

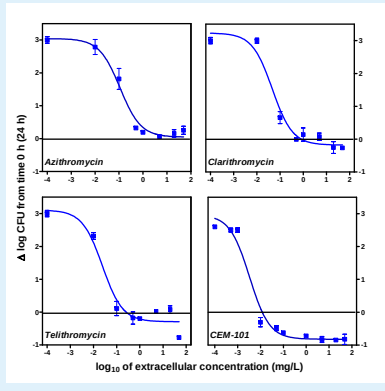
Cond.	AZI	CLR	TEL	CEM-101
Broth pH 7.4				
Cs (mg/L)	~ 3.63	~ 1.41	~ 0.28	~ 0.06
Emax	-1.2 ±0.6	-1.4 ±0.2	-1.0 ±0.4	-1.4 ±0.1
Broth pH 5.5				
Cs (mg/L)	/	~ 10.47	~ 9.33	~ 1.48
Emax	2.1 ±0.1	-1.5 ±0.8	-1.4 ±0.9	-1.6 ±0.4
THP-1				
Cs (mg/L)	~ 10	~ 0.98	~ 0.28	~ 0.02
Emax	0.1 ±0.1	-0.1 ±0.1	-0.4 ±0.1	-0.8 ±0.2

Dose-response studies in Mueller-Hinton broth:



Against *S. aureus* ATCC 25923 and in broth,
- **at pH 7.4, CEM-101 is systematically more active than AZI, CLR and TEL**
- **at pH 5.5, AZI, CLR and TEL show significant decrease of their potencies, while CEM-101 shows less change.**

Dose-response studies in infected THP-1 macrophages



Against intraphagocytic *S. aureus* ATCC 25923, CEM-101
- **is more potent than AZI, CLR and TEL (lower C_s)**
- **is able to reduce the intracellular inoculum (Emax ~ 1 log), which is not the case for the comparators**

REFERENCES

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