Pharmacodynamic evaluation of the Intracellular activity of CEM-101, a novel fluoroketolide, towards *Staphylococcus aureus*, *Listeria monocytogenes*, and *Legionella pneumophila* in human THP-1 macrophages.

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Abstract (revised)

Background:

Macrolides accumulate in eukaryotic cells, which is considered advantageous for the treatment of intracellular infections. Ketolides, i.e. macrolides in which the cladinos has been removed and replace by a ketone function, recover activity against most macrolide-resistant organisms. CEM-101 is a novel fluoroketolide with a 11,12 carbamate-butyl-[1,2,3]-triazolyl-phenylamino side chain, which demonstrates enhanced potency compared to telithromycin. We have assessed the cellular accumulation and intracellular activity of CEM-101 against the intracellular forms of *Staphylococcus aureus (S.a.)*, *Listeria monocytogenes (L.m.)*, and *Legionella pneumophila (L.p.)* in comparison with azithromycin (AZM), clarithromycin (CLR), and telithromycin (TEL).

Methods:

All experiments were performed with the human macrophage cell line THP-1. Drug accumulation was measured using a bioassay. Intracellular activity was measured over time and concentration by following the change in cell-associated CFU compared to post-phagocytosis levels (see details in JAC 2004,54:288-9 [*L.m.* – strain EGD]; AAC 2006, 50:841-51 [*S.a.* strain ATCC 25923]; similar protocol for *L.p.* [strain ATCC 33153]).

Results:

Uptake of CEM-101 was linear over time, reaching accumulation levels about 375-fold within 24 h (AZM, 160 x, CLR, 30 x, TEL, 21 x). Accumulation was suppressed by acid pH or addition of the proton ionophore monensin, but not modified by verapamil or gemfibrozil (preferential inhibitors of P-gp and MRP, respectively). MIC and intracellular activities (developed in a concentration-dependent fashion [Hill equation] are shown in the Table.

	CEM-101			AZM			CLR			TEL		
	MIC [a]	Cs [b]	E_{max} [c]	MIC [a]	Cs [b]	E_{max} [c]	MIC [a]	Cs [b]	E_{max} [c]	MIC [a]	Cs [b]	E_{max} [c]
S.a.	0.06	0.022	-0.86	0.5	> 50	0.04	0.5	0.84	-0.18	0.25	0.63	-0.29
L. m.	0.004	0.11	-0.66	1	11.6	-0.81						
L. p.	0.004	0.018	-1.03	0.016	2.90	-0.83	0.007	0.12	-0.71	0.007	0.06	-0.63

[a] mg/L; [b] static concentration (mg/L) at 24 h; [c] Δ $\log_{10}$ CFU at 24 h compared to the post-phagocytosis inoculum

CEM-101 showed similar or slightly higher relative maximal efficacy (E_{max}) compared to AZM, CLR or TEL, but considerably higher relative potency (lower EC_{50} and C_s), in relation to its lower MIC when expressed on a mass basis (differences in EC_{50} and C_s between drugs largely vanish if data are expressed as multiples of the MIC).

Conclusions: CEM-101 is a fluoroketolide with enhanced cellular accumulation. It shows improved intracellular potency (on a weight basis) in comparison with AZM, CLR and TEL in this in vitro model (mainly due to its larger intrinsic activity [lower MICs] against target organisms). This should lead to enhanced in vivo potency if using doses similar to those of the comparators tested here.

References

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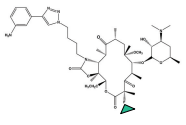
Introduction

Macrolides accumulate inside eukaryotic cells, which has been considered advantageous for the treatment of intracellular infections (1,2), even though we know that these antibiotics express only a minimal fraction of their antibacterial potential intracellularly (3).

11,12-carbamate analogs of clarithromycin carrying a lipophilic side chain show improved activity compared to the parent compound (4). Together with the removal of the cladinose, this led to the discovery and development of the telithromycin (5), the first ketolide to reach clinical approval. CEM-101 is a novel fluoroketolide containing an 11,12-carbamate-butyl-[1,2,3]-triazolyl-aminophenyl side chain (see structure hereunder) that shows enhanced potency compared to telithromycin (6). We have assessed its cellular accumulation and intracellular activity using models that have been developed for the study of the intracellular pharmacodynamics of antibiotics (3,7).

Structural formula of CEM-101

The molecule possesses a 11,12-carbamate-butyl-[1,2,3]-triazolyl-aminophenyl sidechain and a fluorine atom (green arrowhead) substituting position 2 of the macrocycle. The molecule is monocationic at neutral or moderately acidic pH, as the calculated pKa of the aminophenyl group is less than 4 (*vs.* ~ 8.5 for the desosamine).



Methods

Cells: THP-1 myelomonocytic cells (ATCC TIB-202) with macrophage-like activity (7).

Antibiotic assay: microbiological method (7) and accumulation calculated as apparent intracellular/extracellular concentration ratio (7) based on assay of cell protein content (7)

Bacterial strains: *S. aureus* ATCC 25923 (methicillin-sensitive), *L. monocytogenes* strain EGD and *L. pneumophila* strain ATCC 33153

Cell infection and assessment of antibiotic intracellular activities: Time- (0-24h) and concentration-dependent (24 h for *S. aureus* and *L. monocytogenes;* 48h for *L. pneumophila*) experiments as described in ref. 3 (minor adaptations for *L. pneumophila*). Data from concentration-dependent experiments analyzed by non-linear regression using Hill's equation (7) to calculate pharmacological descriptors (E_{max}: maximal reduction of the intracellular inoculum (in $\log_{10}$ units) for an infinitely large antibiotic concentration; E_{min}: increase in intracellular inoculum (in $\log_{10}$ units) for an infinitely low antibiotic concentration; EC_{50}: antibiotic concentration yielding a response halfway between E_{min} and E_{max}; C_s: antibiotic concentration yielding a static effect.

Results

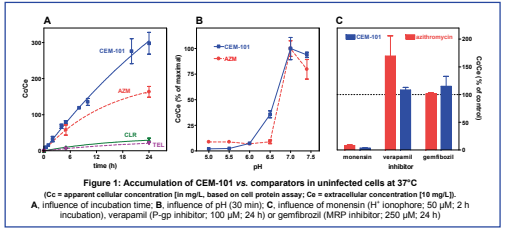


Figure 1: Accumulation of CEM-101 *vs.* comparators in uninfected cells at 37°C
(Cc = apparent cellular concentration [in mg/L, based on cell protein assay; Ce = extracellular concentration [10 mg/L]).
A, influence of incubation time; **B**, influence of pH (30 min); **C**, influence of monensin (H^+ ionophore; 50 μM; 2 h incubation), verapamil (P-gp inhibitor; 100 μM; 24 h) or gemfibrozil (MRP inhibitor; 250 μM; 24 h)

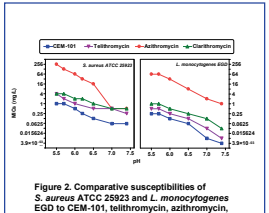


Figure 2. Comparative susceptibilities of *S. aureus* ATCC 25923 and *L. monocytogenes* EGD to CEM-101, telithromycin, azithromycin, and clarithromycin.

MIC determinations in pH-adjusted broth.

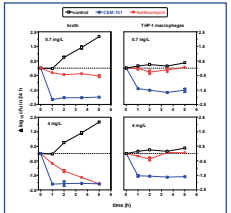


Figure 3: Short-term time-kill effect of CEM-101 and azithromycin towards *S. aureus* (ATCC 25923) in broth vs. in THP-1 macrophages.

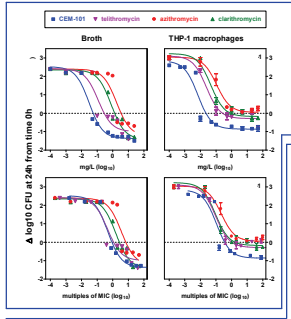


Figure 4: Concentration-effect relationships for CEM-101, telithromycin, clarithromycin, and azithromycin against *S. aureus* (ATCC 25923) in broth or in THP-1 macrophages.

Ordinate: change in CFU (Δ $\log_{10}$ CFU) per mL (broth) or per mg of cell protein (THP-1 macrophages) at 24 h compared to initial inoculum;
Abscissa: weight concentrations (upper rows) or multiples of MIC (measured at pH 7.4; lower rows).

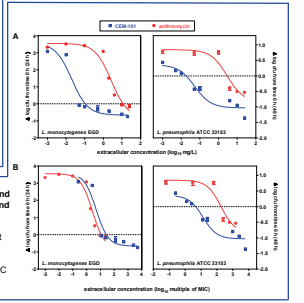


Figure 5: Concentration-effect relationships for CEM-101 and azithromycin towards intraphagocytic *L. monocytogenes* and *L. pneumophila*.

Ordinate: change in CFU (Δ $\log_{10}$ CFU) per mg of cell protein at 24 h (*L. monocytogenes*) or 48 h (*L. pneumophila*) compared to initial post-phagocytosis inoculum;
Abscissa: weight concentrations (upper rows) or multiples of MIC (measured at pH 7.4; lower rows).

Pharmacological parameters of the dose response of *S. aureus* to the antibiotics tested in this study
(data from Figure 4; E_{max}: Δ log CFU compared to post-phagocytosis inoculum; EC_{50} and C_s: mg/L or x MIC)

antibiotic	broth			THP-1 macrophages		
	E_{max}	EC_{50}	C_s	E_{max}	EC_{50}	C_s
CEM-101	-1.37	mg/L 0.03	0.06	-0.86	mg/L 0.0068	0.022
		x MIC 0.48	0.88		x MIC 0.11	0.35
telithromycin	-1.00	mg/L 0.12	0.29	-0.29	mg/L 0.024	0.63
		x MIC 0.46	0.96		x MIC 0.097	1.04
azithromycin	-1.23	mg/L 1.78	3.4	0.04	mg/L 0.11	> 50
		x MIC 3.55	6.87		x MIC 0.22	> 100
clarithromycin	-1.41	mg/L 0.80	1.32	-0.18	mg/L 0.046	0.84
		x MIC 1.59	2.65		x MIC 0.093	1.68

Conclusions

- CEM-101 markedly accumulates in THP-1 macrophages by a proton-dependent mechanism and is not subject to P-gp efflux
- CEM-101 showed significantly greater potency against phagocytized *S. aureus, L. monocytogenes* and *L. pneumophila*, which is entirely attributable to its lower MIC but not to its accumulation.
- CEM-101 should show enhanced *in vivo* potency if used at doses similar to those of the comparators tested here.

This poster will be made available for download after the meeting at http://www.facm.ucl.ac.be/posters.htm

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