

ABSTRACT

Background: Macrolides accumulate in macrophages by diffusion/segregation thanks to their weak basic character and the acidic pH prevailing in phagolysosomes. However, this can be partly defeated by active efflux, as shown for azithromycin and erythromycin in macrophages in which both antibiotics are substrates of the P-glycoprotein. We have examined CEM-101, a novel macrolide/ketolide antibiotic, for intracellular accumulation and activity, and their modulation by P-gp and MRP inhibitors, in comparison with azithromycin.

Methods: Human THP-1 macrophages were used throughout. Accumulation was measured by microbiological assay. Intracellular activity was determined against phagocytized *S. aureus* (ATCC 25923; MICs : CEM-101, 0.125 mg/L; azithromycin, 0.5 mg/L) using a dose-response approach (AAC 2006;50:841-51). Verapamil (100 μM) and gemfibrozil (250 μM) were used as inhibitors of P-glycoprotein and MRP, respectively (AAC, 2007;51:2748-57).

Results: Accumulations and activities after 24 h incubation, with and without efflux transporters inhibitors, are shown in Table

Conditions	Azithromycin			CEM-101		
	Cc/Ce (24h) ¹	Intracellular activity (Δ log cfu at 24 h)		Cc/Ce (24h) ¹	Intracellular activity (Δ log cfu at 24 h)	
		Static dose (mg/L)	E _{max} ²		Static dose (mg/L)	E _{max} ²
Control	127.7 ± 23.5	~ 7.0	0.10 ± 0.09	268.1 ± 7.1	~ 0.02	- 0.85 ± 0.23
Verapamil	216.37 ± 46.6	~ 0.2	- 0.37 ± 0.15	290.2 ± 12.9	~ 0.03	-0.59 ± 0.22
Gemfibrozil	129.12 ± 2.69	~ 3.8	-0.12 ± 0.20	308.2 ± 47.8	~ 0.03	-0.73 ± 0.20

¹ apparent cellular to extracellular concentration ratio

² maximal decrease of intracellular cfu compared to post-phagocytosis inoculum (calculated from non-linear regression [sigmoidal] of dose-effect response experiments)

Conclusions:

In comparison with AZM, CEM-101 showed a larger intracellular accumulation and intracellular activity than AZM. This was not affected by P-gp or MRP-inhibitors suggesting that CEM-101, in contrast to AZM, is not a substrate of the corresponding eucaryotic efflux transporters.

BACKGROUND AND AIM

• Active efflux in eukaryotic cells, is now recognized as a key determinant in the modulation of the pharmacokinetic and pharmacodynamic properties of antibiotics. Earlier studies with azithromycin have revealed that this organic weak base drug is the substrate of P-gp-mediated efflux, which partially defeats its cellular accumulation and activity towards intracellular pathogens such as *S. aureus*.¹

• In the present study, we aimed at investigating the role of active efflux in the modulation of the cellular accumulation and intracellular activity of CEM-101 (also termed as OPT-1068), a novel macrolide/ketolide under investigation and showing marked activity against macrolide-resistant *S. aureus*.

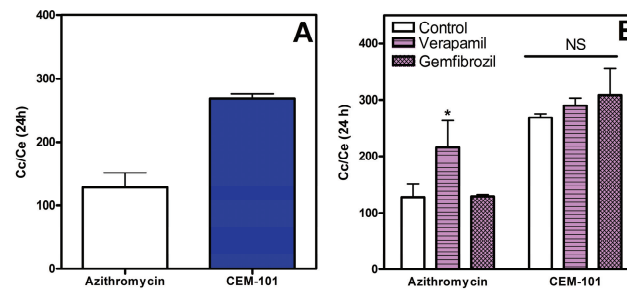
MATERIAL AND METHODS

CELLULAR ACCUMULATION OF ANTIBIOTICS. The cellular content in macrolides was measured in THP-1 macrophages² by microbiological assay, using *S. aureus* ATCC 25923 as test organism. Cell proteins was assayed in parallel using the Folin-Ciocalteu/Biuret method. The cell associated content in macrolides was expressed by reference to the total cell protein content, and converted into apparent concentrations using a conversion factor of 5 μL per mg of cell protein (as commonly used for cultured cells).

INTRACELLULAR ACTIVITY OF ANTIBIOTICS. The determination of antibiotic activity against intraphagocytic *S. aureus* strain ATCC 25923 was determined exactly as described earlier⁽³⁻⁵⁾.

RESULTS

A) CELLULAR ACCUMULATION



• We first measured the cellular accumulation of CEM-101 in comparison with that of azithromycin in THP-1 cells (panel A).

At 24 h, both antibiotics concentrate to large extents in cells, but with a significantly larger value (Cc/Ce) for CEM-101.

• In a second stage, we investigated whether CEM-101 is a substrate of P-gp or MRP efflux transporters (panel B).

Using a P-gp (verapamil) or MRPs inhibitor (gemfibrozil), no significant variations of the cellular accumulation of CEM-101 are observed while verapamil increases significantly the cellular accumulation of azithromycin.

B) INTRACELLULAR ACTIVITY

Full dose-responses studies were performed to assess the impact of of active efflux in the modulation of the intracellular activity of CEM-101 and azithromycin against intraphagocytic *S. aureus* (strain ATCC 25923 [MICs: CEM-101, 0.125 mg/L; Azithromycin, 0.5 mg/L])

Antibiotics were compared at 24h for:

- (i) their relative static concentration (C_s), and
- (ii) their relative maximal efficacy (E_{max})

A) STATIC CONCENTRATION (C _s [in mg/L])		
Conditions	Azithromycin	CEM-101
Control	~ 7.0	~ 0.02
Verapamil	~ 0.2	~ 0.03
Gemfibrozil	~ 3.8	~ 0.03

B) RELATIVE MAXIMAL EFFICACY (E _{max})		
Conditions	Azithromycin	CEM-101
Control	0.10 ± 0.09	- 0.85 ± 0.23
Verapamil	- 0.37 ± 0.15	-0.59 ± 0.22
Gemfibrozil	-0.12 ± 0.20	-0.73 ± 0.20

While verapamil (but not gemfibrozil) increases the intracellular activity of azithromycin, neither inhibitor have significant effect on the activity of CEM-101, suggesting that the latter, in contrast with azithromycin, is not a substrate of the corresponding eukaryotic transporters.

CONCLUSIONS

CEM-101, a novel macrolide/ketolide in development, shows :

- extensive uptake by THP-1 macrophages
- more intense intracellular activity towards phagocytized *S. aureus* (ATCC 25923) as compared to azithromycin
- No detectable recognition by P-gp or MRPs transporters (based on observations with inhibitors), in contrast to azithromycin (known substrate of P-gp transporters in THP-1 macrophages)

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Acknowledgments

We are grateful to M.-C. Cambier for its dedicated assistance. S.L. was Boursière of the Belgian *Fonds pour la Recherche dans l'Industrie et l'Agriculture* (FRIA) and is now a *Chargée de Recherches* of the Belgian *Fonds de la Recherche Scientifique* (F.R.S.-F.N.R.S.). F.V.B. is *Maître de Recherches* of the Belgian *Fonds de la Recherche Scientifique* (F.R.S.-F.N.R.S.). This work was supported by a grant-in-aid from Cempra Pharmaceuticals.