ABSTRACT

Background: Macrolides accumulate intracellularly by efflux-regulation 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 and 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 (200 µM) were used as inhibitors of P-glycoprotein and MRP, respectively (AAC, 2007;51:2746-57).

Results: Accumulations and activities after 24 h incubation, with and without efflux transporters inhibitors, are shown in Table 1. 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). No significant variations of the cellular accumulation of CEM-101 are observed while verapamil increases significantly the cellular accumulation of azithromycin.

Conclusions: 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.

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 macrophages was measured in THP-1 macrophages by microbiological assay, using S. aureus ATCC 25923 as test organism. Cell proteins were assayed in parallel using the Folin-Ciocalteu/Bluer method. The cell associated content in macrophages 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.

REFERENCES


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

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