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Antimicrobial activity of the fluoroketolide solithromycin (CEM-101) against *L. pneumophila*

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Abstract

Objectives: The goal of this study was to evaluate the *in vitro* and the intra-cellular activity of solithromycin, a fluoroketolide, against clinical *Legionella pneumophila* serogroup 1 (Lp1) strains collected in Ontario, Canada.

Methods: A total of 196 clinical Lp1 isolates collected from 1980 to 2011 and previously sequence-base typed at the Public Health Ontario Laboratories, Toronto, Canada, were studied. *In vitro* activity of solithromycin was compared to azithromycin using the broth microdilution method. To investigate the intracellular activity of solithromycin, *in vitro* invasion assays were performed using monolayers of NCI-H292 lung epithelial cells and 18 clinical strains displaying different azithromycin susceptibility profiles. Infected cultures were treated with solithromycin or azithromycin and the intracellular activity of each antibacterial agent was determined by counting viable intracellular bacteria after 24 and 48 hours of exposure.

Results: Solithromycin displayed a MIC_{50} of ≤0.015 µg/ml and a MIC_{90} of 0.031 µg/ml, making its activity 8-fold and 32- fold higher than azithromycin, respectively. 99% of the isolates presented MICs for solithromycin ranging from ≤0.015 µg/ml to 0.031 µg/ml whereas 83.6% of the isolates showed MICs for azithromycin ranging from 0.062 µg/ml to 0.25 µg/ml. Interestingly 96.7% of the clinically prevalent *L. pneumophila* sequence type 1 isolates were identified with higher MICs ranging from 0.5 µg/ml to 2 µg/ml compared to others sequence types. Moreover solithromycin and azithromycin both inhibited growth of all Lp1 strains in lung epithelial cells at 1X or 8X MICs, displaying bacteriostatic effects rather than a bactericidal activity.

Conclusion: Solithromycin demonstrated the highest *in vitro* potency against all *L. pneumophila* isolates when compared to azithromycin. This new antimicrobial agent may prove to be an effective treatment option for *L. pneumophila* infections if clinical trials demonstrate efficacy and safety in adult indications. This is particularly relevant in times when strains resistant to macrolides and fluoroquinolones are emerging worldwide, complicating the antibiotic treatment of intracellular pathogens.

Introduction and Objectives

Legionellosis is a major public health concern in industrialized countries. Manifestations of this disease range from a mild respiratory illness to a severe and rapidly fatal pneumonia.[1] The case fatality rate of legionellosis ranges between 40 to 80% in untreated immuno-suppressed patients, but can be reduced from 5 to 30% with appropriate case management.[2,3]
Macrolides and quinolones have become the preferred and recommended agents in the treatment of Legionnaire's disease.[4-6] However, development of resistance in *L. pneumophila* has been reported [6,7] and only sporadically investigated.[8,9] Development of resistance *in vitro* by exposure to erythromycin, ciprofloxacin and rifampicin have been also reported.[10] New therapeutic options are needed to counteract the emergence of resistance threatening the use of first-choice antimicrobials.
Solithromycin (CEM-101), which is a new fluoroketolide developed in recent years, demonstrates high potency against Gram-positive and some Gram-negative pathogens.[11] Moreover, solithromycin is currently in Phase 3 clinical trials for community bacterial pneumonia. Our objective was to evaluate the *in vitro* activity of solithromycin compared to azithromycin against *L. pneumophila* collected from 1980 to 2011, in Ontario (Canada). In addition, we also investigated the intracellular activity of solithromycin using monolayers of NCI-H292 lung epithelial cells with a subset of selected strains.

Materials and Methods

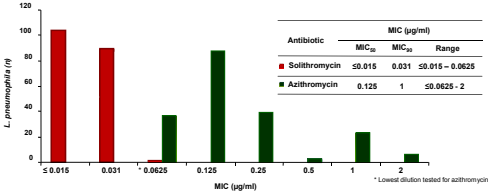
Strains
Since 1978, the diagnosis of Legionnaire's disease has been centralized at the Public Health Ontario Laboratory (PHO), which serves as the provincial reference laboratory and performs all testing for outbreak investigations and most testing of clinical specimens in Ontario. A total of 196 *L. pneumophila* serogroup 1 (Lp1) clinical isolates were analyzed in this study. They were collected from 1980 to 2011, representative of the strains isolated in Ontario in the past 3 decades and previously sequence-base typed at the PHO.[12] Isolates were stored in trypticase soy broth supplemented with 5% horse blood at -80°C . Cultures from the frozen stock were prepared by inoculating buffered charcoal yeast extract (BCYE) plates. Plates were incubated for 3 days at 37°C in 5% CO_2.

Determination of Minimum inhibitory concentration (MIC)
Colonies from all isolates were sub-cultured on BCYE plates for 3 days at 37°C in 5% CO_2 before the antimicrobial testing was performed. MICs of solithromycin and azithromycin were determined by the broth microdilution method in 96-well microtitre plates according to the Clinical and Laboratory Standards Institute guidelines[13] with minor modifications.[14] MICs were read as the first well showing no visible growth after 48h incubation at 37°C in 5% CO_2.

Intracellular activity of solithromycin
Gentamicin protection assays were performed using monolayers of NCI-H292 human lung epithelial cells and 18 Lp1 clinical isolates displaying azithromycin susceptibility levels ranging from 0.0625 µg/ml to 2 µg/ml. NCI-H292 cells were infected at an Multiplicity of Infection (MOI) of 30:1 for 3 hours. After internalization, the bacteria cells were exposed to solithromycin at 1X and 8X the MICs of each strain. Wells containing no antibiotic were used as growth controls at each time point (0 and 24 h). At indicated time points, lysed cells and their respective supernatants were pooled and homogenized by pipetting. The intracellular activity of each antibacterial agent was determined by counting CFU/ml on each plate. All assays were performed in quadruplicate with two independent biological duplicates. Results were expressed as percentage of viability defined as total *Legionella* CFU at 24h with agent divided by total *Legionella* CFU at 0h without agent x 100. Values ≥100% indicate absence of antimicrobial inhibition, whereas values <100% indicate an inhibitory effect.

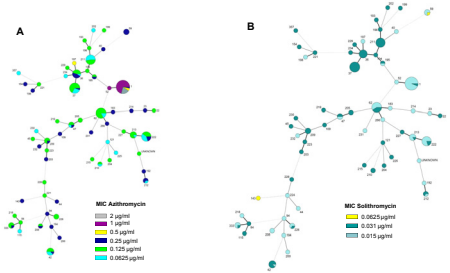
Results

Figure 1. *In vitro* activity and MIC distribution of solithromycin and azithromycin against Lp1 clinical isolates (n=196)



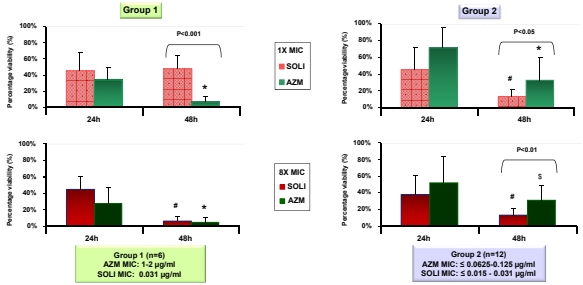
Antibiotic	MIC (µg/ml)		
	MIC_{50}	MIC_{90}	Range
Solithromycin	≤0.015	0.031	≤0.015 – 0.0625
Azithromycin	0.125	1	≤0.0625 - 2

Figure 2. Minimum spanning tree of Lp1 clinical isolates (n=196)



Antimicrobial susceptibilities and categorical clustering based on sequence types (ST). STs sharing the maximum number of single locus variants were connected first. Each circle represents a ST, the size of which is proportional to the number of isolates within that particular type. Colors within circles indicate the MIC ranges for A. Azithromycin and B. Solithromycin. The tree was generated using the Bionumerics software.

Figure 3. Intracellular activity of solithromycin (SOLI) compared to azithromycin (AZM) against Lp1 clinical isolates



Conclusions

- Solithromycin has better *in vitro* activity than azithromycin against a variety of *L. pneumophila* clinical isolates displaying azithromycin susceptibility levels ranging from ≤ 0.0625 µg/ml to 2 µg/ml.
- Solithromycin has a MIC_{50} of ≤ 0.015 µg/ml and a MIC_{90} of 0.031 µg/ml, making its activity 8-fold and 32-fold higher than azithromycin, respectively.
- 96.7% of ST1 isolates (commonly associated with Legionnaire's disease and dispersed worldwide[15]) were associated with higher MICs to azithromycin (ranging from 0.5 to 2 µg/ml) and low MICs to solithromycin (0.031 µg/ml) compared to other sequence types.
- 93.7% of the strains belonging to the major clonal group including ST1 isolates were also associated with higher MICs to azithromycin (ranging from 0.5 to 2 µg/ml) and low MICs to solithromycin (0.031 µg/ml). These results suggest that the predominant clonal group may have acquired some resistance mechanism affecting the activity of macrolides but not of solithromycin.
- In intracellular assays, solithromycin inhibited the growth of Lp1 clinical strains independently of their sequence type, and maintained the same efficiency as azithromycin after 24 h of exposure. Interestingly, after extending the incubation time to 48h, solithromycin showed a much higher relative potency on strains presenting lower MICs to azithromycin (up to 64 and 4 times lower for Group 1 and Group 2, respectively).
- Solithromycin demonstrated the highest *in vitro* potency against all *L. pneumophila* isolates when compared to azithromycin, making it a potential future option for legionellosis treatment if clinical trials demonstrate efficacy and safety in adult indications.

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