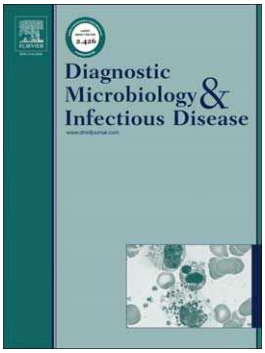

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PII: S0732-8893(17)30285-7
DOI: doi: 10.1016/j.diagmicrobio.2017.09.011
Reference: DMB 14429

To appear in: *Diagnostic Microbiology and Infectious Disease*

Received date: 10 March 2017
Revised date: 23 June 2017
Accepted date: 16 September 2017

Please cite this article as: Huang David B, Duncan Leonard R, Flamm Robert K, Dryden Matthew, Corey G. Ralph, Wilcox Mark H, Torres Antoni, File Jr Thomas M, The Effect of Pulmonary Surfactant on the *In Vitro* Activity of Iclaprim Against Common Respiratory Bacterial Pathogens, *Diagnostic Microbiology and Infectious Disease* (2017), doi: 10.1016/j.diagmicrobio.2017.09.011

The Effect of Pulmonary Surfactant on the In Vitro Activity of Iclaprim Against Common Respiratory Bacterial Pathogens

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Abstract

The in vitro antimicrobial activity of iclaprim, a novel diaminopyrimidine, against common respiratory bacteria remained unchanged in the presence of pulmonary surfactant (Survanta®) at concentrations that greatly antagonized the antimicrobial activity of daptomycin. These results indicate that iclaprim could be a potential treatment for pneumonia caused by susceptible and multidrug resistant bacteria.

Keywords: iclaprim, surfactant, pneumonia, in vitro

1. Introduction

Iclaprim represents a novel diaminopyrimidine, which inhibits bacterial dihydrofolate reductase (DHFR) and is active against emerging drug-resistant pathogens (Sader et al., 2009; Schneider et al., 2003). Iclaprim exhibits potent in vitro activity against Staphylococcus aureus, Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis and atypical bacteria (i.e., Legionella pneumophila, Chlamydia pneumoniae, and Mycloplasma pneumoniae) that cause pneumonia (Sader et al., 2009; Morrissey et al., 2009). Iclaprim demonstrates rapid in vitro bactericidal activity in time kill studies in human plasma (Laue et al., 2009). Because of these findings, iclaprim is potentially well suited for treating patients with nosocomial pneumonia caused by susceptible and multidrug resistant pathogens. In the present study, we investigated the effect of bovine pulmonary surfactant (BPS), a major component of epithelial lining fluid, on the antibacterial activity of iclaprim against common Gram-positive and Gram-negative respiratory bacteria in vitro.

2. Methods

2.1 Collection of bacterial isolates

Clinical isolates were identified by the submitting laboratories and confirmed by JMI Laboratories using standard bacteriologic algorithms and methodologies, including Matrix-Assisted Laser Desorption Ionization-Time Of Flight Mass Spectrometry (MALDI-TOF MS). When necessary, MALDI-TOF MS was performed using the Bruker Daltonics MALDI Biotyper (Billerica, Massachusetts, USA), following manufacturer's instructions. Isolates selected were from bacterial species commonly associated with pneumonia. The clinical isolates were S.

pneumoniae (n=2), H. influenzae (n=1), M. catarrhalis (n=2), S. aureus (n=1), and Klebsiella pneumoniae (n=1). Table 2 shows the seven American Type Culture Collection (ATCC) reference strains that were tested. The 14 isolates and strains in this study were similar to the numbers examined in prior studies of the effect of surfactant on in vitro activity of antimicrobials (Dallow et al, 2014 (n=18); Glacobbe et al 2017 (n=7); Gotfried et al, 2008 (n=2)). The specific isolates were chosen because they were recent clinical isolates from species associated with pulmonary infections; the reference strains are frequently used ATCC QC reference strains.

2.2 Susceptibility testing

Antibacterial susceptibility testing was measured by JMI Laboratories (North Liberty, Iowa, USA). The seven nonduplicative, nonconsecutive clinical isolates were collected from US (n=4), Mexico (n=1), and Italy (n=2). Susceptibility testing was performed by broth microdilution in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines M07-A10 (2015) and the standard operating procedures at JMI Laboratories. Minimum inhibitory concentrations (MICs) were based on CLSI criteria (2015). There are no published breakpoints for iclaprim, which is typical for drugs in development. E. coli, K. pneumoniae, M. catarrhalis, E. faecalis, and S. aureus were tested in cation-adjusted Mueller-Hinton broth (CA-MHB). S. pneumoniae was tested in CA-MHB supplemented with 2.5-5% lysed horse blood, and H. influenzae was tested in Haemophilus Test Medium. Quality control ranges and interpretation of results were performed in accordance with CLSI M100-S26 (2016) methods. QC ranges for iclaprim were those approved by CLSI and published in M100-S26 (2016).

Because bovine pulmonary surfactant can introduce cloudiness to the MIC testing media,

antimicrobial growth inhibition was also evaluated using the colorimetric metabolic indicator resazurin (Camlab Ltd., Cambridge, UK). Following visual MIC value determinations, 10 µL of a resazurin solution (6.75 – 7.0 mg/mL in H_2O) was added to the test wells in each panel, and the panels were incubated for an additional 1-3 hours at 35˚C in ambient atmosphere (Elshikh et al., 2016; Sarker et al., 2007). S. pneumoniae panels were omitted from resazurin analysis because the color change was obscured by the lysed horse blood present in the test medium. Growth inhibition was then evaluated as a visible color change from blue (no growth) to pink (robust growth) and recorded as a MIC_{RZ} value.

2.3 Pulmonary surfactant interaction

The MICs of iclaprim, levofloxacin, and daptomycin were measured against Gram-positive and Gram-negative isolates. Daptomycin was included as a positive control and levofloxacin was included as a negative control because published data showed an increase in daptomycin MICs but no increase in levofloxacin MICs in the presence of pulmonary surfactant (Silverman et al, 2005; Giacobbe et al, 2017).

A new vial of bovine pulmonary surfactant (BPS; Survanta®; Abbott Laboratories, Columbus, OH) was utilized for each independent experiment. Each vial was mixed thoroughly and a 100 µL aliquot was spread on an agar growth plate, which was incubated overnight to confirm sterility. BPS was added to the MIC test medium to a final concentration of 2.5% (v/v). The concentration of surfactant was expressed in terms of percent volume of Survanta® suspension, which consisted of phospholipid (25 mg/mL) and surfactant proteins (<1 mg/mL) in 0.9% sodium chloride solution (Goerke, 1998). The concentration of 2.5% was chosen because the effect of BPS on S. aureus daptomycin MIC values plateaus above approximately 1%

(Silverman et al, 2005; Dallow et al, 2014; Gotfriend et al, 2008) and because MIC values become difficult to read using CLSI methodology above 2.5%. MICs of daptomycin were determined in CAMHB with the Ca^{2+} content adjusted to 50 mg/L.

3. Results

3.1 Pulmonary surfactant interaction

Table 1 shows the MIC values of iclaprim, daptomycin, and levofloxacin with or without resazurin in the presence and absence of BPS. Where applicable, all MICs for ATCC reference strains were within the ranges published by CLSI (CLSI, 2016) with the exception of iclaprim against E. coli ATCC 25922, where the MICs values were one two-fold dilution below the published QC range (Table 1). The MIC and MIC_{RZ} values for all drug/isolate combinations agreed well in both the absence and presence of 2.5% (v/v) BPS. The presence of BPS had minimal or no effect on the MIC and MIC_{RZ} values of iclaprim for any of the tested strains or isolates. Most MICs and MIC_{RZ} values were unchanged, and where shifts were observed, these were only one drug dilution. In contrast, the MIC and MIC_{RZ} values of daptomycin against the respiratory Gram-positive reference strains and clinical isolates increased 16 to 128-fold to ≥16 µg/mL in the presence of 2.5% BPS, consistent with published data (Dallow et al., 2014; Silverman et al. 2005). As expected, the presence of BPS had little or no effect on the MIC and MIC_{RZ} values of levofloxacin (see Table 1).

4. Discussion

In summary, this report demonstrates that iclaprim is active in vitro against common respiratory bacterial pathogens (S. pneumoniae, H. influenzae, S. aureus, K. pneumoniae and M. catarrhalis) even in the presence of pulmonary surfactant. In contrast, the inhibitory effect of surfactant on antibacterial activity was observed with daptomycin. This inhibitory MIC effect of surfactant on daptomycin activity has been reported to be mediated by binding of surfactant components to specific structures present on antibiotics, such as the the lipophilic side-chain of daptomycin (Silverman et al., 2005). Thus, unlike daptomycin, the potency of iclaprim against common respiratory bacterial pathogens and the absence of antagonism by pulmonary surfactant against iclaprim shown in this in vitro study suggests that iclaprim should be active against pulmonary pathogens in pneumonia in vivo.

A Phase 1 study investigated the tissue distribution of a single IV dose of iclaprim in relevant lung compartments (Andrews et al, 2007). Iclaprim concentrations were found in epithelial ling fluid (ELF) and alveolar macrophages (AM), up to 20- and 40-fold higher, respectively, than in plasma. In addition, iclaprim concentrations in plasma, ELF and AM after a single IV dose of 1.6 mg/kg exceeded iclaprim MICs for penicillin- susceptible S. pneumoniae (MIC_{90} 0.06 mg/L) and methicillin-resistant S. aureus (MIC_{90} 0.12 mg/L) for up to 7 hours; mean iclaprim concentrations in ELF exceeded the iclaprim MICs observed for S. pneumoniae with intermediate penicillin resistance (MIC_{90} 2 mg/L) and full resistance (MIC_{90} 4 mg/L) for up to 7 and 4 hours, respectively.

A Phase 2 study comparing the clinical cure rates of two iclaprim dosages with vancomycin in the treatment of patients with nosocomial pneumonia suspected or confirmed to be caused by Gram-positive pathogens showed iclaprim and vancomycin to have comparable clinical cure rates and safety profiles (Huang et al., submitted). The cure rates in the intent-to-

treat population were 73.9% (17 of 23), 62.5% (15 of 24), and 52.2% (12 of 23) at the post-treatment test of cure visit in the iclaprim 0.8 mg/kg intravenous (IV) q12h, iclaprim 1.2 mg/kg IV q8h, and vancomycin 1 g IV q12h groups, respectively (iclaprim q12h versus vancomycin $p = 0.13$; and iclaprim q8h versus vancomycin $p = 0.47$). The death rates within 28 days of the start of treatment were 8.7% (2 of 23), 12.5% (3 of 24), and 21.7% (5 of 23) for the iclaprim q12h, iclaprim q8h, and vancomycin groups, respectively (no statistically significant differences). Collectively, the current in vitro study, and previous Phase 1 and 2 studies support that iclaprim could be a potential treatment for pneumonia, including nosocomial pneumonia caused by susceptible and multidrug resistant Gram-positive bacteria.

Table 1 In vitro activity of iclaprim, daptomycin and levofloxacin in the presence and absence of 2.5% bovine pulmonary surfactant

			Iclaprim MIC (µg/mL) (80% read)				Daptomycin MIC (µg/mL)				Levofloxacin MIC (µg/mL)			
Species	**Source**	**Strain**	**CLSI**	**CLSI BPS**	**Resazurin**	**Resazurin BPS**	**CLSI**	**CLSI BPS**	**Resazurin**	**Resazurin BPS**	**CLSI**	**CLSI BPS**	**Resazurin**	**Resazurin BPS**
E. coli	ATCC	25922	0.5	0.5	1	1	ND[a]	ND	ND	ND	0.015	0.03	0.015	0.03
E. faecalis	ATCC	29212	≤0.015	≤0.015	≤0.015	≤0.015	2	>16	2	>16	1	1	1	1
E. faecalis	ATCC	33186	≤0.015	≤0.015	0.03	≤0.015	1	>16	1	>16	4	1	4	2
H. influenzae	ATCC	49247	0.12	0.12	0.25	0.25	ND	ND	ND	ND	0.03	0.03	0.03	0.03
H. influenzae	Clinical isolate	824704	0.06	0.06	0.06	0.06	ND	ND	ND	ND	0.015	0.015	0.015	0.015
K. pneumoniae	ATCC	700603	4	4	4	4	ND	ND	ND	ND	1	1	1	1
K. pneumoniae	Clinical isolate	858055	2	2	2	2	ND	ND	ND	ND	0.06	0.12	0.06	0.06
M. catarrhalis	Clinical isolate	893806	4	ND	4	8	8	ND	8	>16	0.06	ND	0.06	0.06
M. catarrhalis	Clinical isolate	893807	4	ND	4	4	8	ND	8	>16	0.06	ND	0.06	0.06
S. aureus	ATCC	29213	0.06	0.06	0.06	0.06	0.5	>16	0.5	>16	0.25	0.12	0.25	0.12
S. aureus	Clinical isolate	825189	0.06	0.12	0.06	0.12	0.25	>16	0.25	>16	0.25	0.25	0.25	0.25

S. pneumoniae	ATCC	49619	0.06	0.06	ND	ND	0.25	16	ND	ND	1	1	ND	ND
S. pneumoniae	Clinical isolate	818757	0.06	0.06	ND	ND	0.25	16	ND	ND	1	1	ND	ND
S. pneumoniae	Clinical isolate	825175	0.12	0.12	ND	ND	0.25	16	ND	ND	1	1	ND	ND

[a]ND, not done (resazurin color change was difficult to interpret in the presence of blood or daptomycin was inactive against Gram-negative bacteria, or M. catarrhalis growth was difficult to interpret in the presence of BPS)

Abbreviations: MIC, minimal inhibitory concentration; CLSI, Clinical and Laboratory Standards Institute; BPS, bovine pulmonary surfactant (Survanta®, tested at 2.5% v/v); ATCC, American Type Culture Collection

Source of Funding and Conflict of Interest

This study was supported by Motif BioSciences Inc., New York, USA.

DBH is an employee of Motif BioSciences. LRD and RKF are employees of JMI Laboratories. JMI Laboratories contracted to perform services in 2016 for Achaogen, Actelion, Allecra Therapeutics, Allergan, AmpliPhi Biosciences, API, Astellas Pharma, AstraZeneca, Basilea Pharmaceutica, Bayer AG, BD, Biomodels, Cardeas Pharma Corp., CEM-102 Pharma, Cempra, Cidara Therapeutics, Inc., CorMedix, CSA Biotech, Cutanea Life Sciences, Inc., Debiopharm Group, Dipexium Pharmaceuticals, Inc., Duke, Entasis Therapeutics, Inc., Fortress Biotech, Fox Chase Chemical Diversity Center, Inc., Geom Therapeutics, Inc., GSK, Laboratory Specialists, Inc., Medpace, Melinta Therapeutics, Inc., Merck & Co., Micromyx, MicuRx Pharmaceuticals, Inc., Motif Bio, N8 Medical, Inc., Nabriva Therapeutics, Inc., Nexcida Therapeutics, Inc., Novartis, Paratek Pharmaceuticals, Inc., Pfizer, Polyphor, Rempex, Scynexis, Shionogi, Spero Therapeutics, Symbal Therapeutics, Synlogic, TenNor Therapeutics, TGV Therapeutics, The Medicines Company, Theravance Biopharma, ThermoFisher Scientific, VenatoRx Pharmaceuticals, Inc., Wockhardt, Zavante Therapeutics, Inc. There are no speakers' bureaus or stock options to declare. TMF has served as a consultant for Motif BioSciences, Allergan, Medicines Company, Merck, Nabriva, Paratek, and Cempra. AT has served as a consultant for Motif BioSciences. AFS has served as a consultant to, received research support from, or been a speaker for Abbott, Actavis, Alios, Astellas, AstraZeneca, Bayer, BMS, Cardeas, Medicines Company, Merck, Pfizer, Roche, Tetraphase, Theravance, and Wockhardt Pharma. MHW has received consulting fees from Abbott Laboratories, Actelion, Astellas, Astra-Zeneca, Bayer, Biomèrieux, Cerexa, Cubist, Durata, The European Tissue Symposium,

The Medicines Company, MedImmune, Merck, Motif Biosciences, Nabriva, Optimer, Paratek, Pfizer, Qiagen, Roche, Sanofi-Pasteur, Seres, Summit, and Synthetic Biologics; lecture fees from Abbott, Alere, Astellas, Astra-Zeneca, Merck, Pfizer & Roche; grant support from Abbott, Actelion, Astellas, Biomèrieux, Cubist, Da Volterra, MicroPharm, Morphochem AG, Sanofi-Pasteur, Seres, Summit and The European Tissue Symposium, Merck.

PH is a former employee of Arpida. MD has received speaker's and/or consultancy fees from AstraZeneca, Bayer, Janssen-Cilag, Motif BioSciences, Novartis, Pfizer, and Merck, Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc. R.C. has received consultancy fees from Cempra Pharmaceuticals, PRA International, Furiex Pharmaceuticals, Inimex Pharmaceuticals, Dr. Reddy's Laboratories, Cubist Pharmaceuticals, Cerexa/Forest Laboratories, AstraZeneca, GlaxoSmithKline, Pfizer, Merck, Trius Therapeutics, ContraFect, Theravance, and Astellas Pharma and served on an advisory board for Pfizer, Polymedix, Trius Therapeutics, Rib-x Pharmaceuticals, Seachaid Pharmaceuticals, BioCryst Pharmaceuticals, Durata Therapeutics, Achaogen, Gilead Sciences, ContraFect, Cempra, and Nabriva Therapeutics. R.C. received research grants from Theravance, Innocoll, and The Medicines Company.

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Highlights

- Iclaprim is active in vitro against common respiratory pathogens.
- The in vitro activity of iclaprim is unchanged in pulmonary surfactant.
- Iclaprim may be a treatment for pneumonia, including nosocomial pneumonia.