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Accumulation of the Pleuromutilin Antibiotic BC-3781 in Murine Macrophages and Effect of Lung Surfactant on the BC-3781 *In Vitro* Activity

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ABSTRACT

Background: BC-3781 is a pleuromutilin antibiotic that has completed Phase 2 studies in ABSSSI. The PK of BC-3781 in the lung and its activity against organisms typically associated with CABP suggest a potential for study in this indication. The uptake of BC-3781 in macrophages was investigated since the targeted delivery of active drug to infection sites by phagocytes may be beneficial for the activity at localized areas of infection. In addition, the effect of bovine lung surfactant (Survanta™) on the BC-3781 MIC was evaluated.

Methods: The intracellular concentration (Ci) of BC 3781 in J774 murine macrophages was determined in triplicate by LC-MS/MS at extracellular concentrations (Ce) of 1 and 5 µg/ml over 1 - 5 h of incubation. Azithromycin and penicillin served as positive and negative controls.

The antibacterial activity of BC-3781 was evaluated by checkerboard broth microdilution in the presence of Survanta™ at 0.06-4% (v/v) against multi-drug resistant and wild-type *S. pneumoniae* (*n* = 3), *S. aureus* (MRSA and MSSA, *n = 2*), *H. influenzae* (*n* = 2) and *β* -lactamase producing *E. coli* (*n = 1*).

Results: BC-3781 showed approximately 50-fold accumulation in macrophages with a Ci/Ce ratio of 47.7 and 50.9 at a Ce of 1 and 5 µg/mL, respectively (t = 5 h). Significant Ci of 35.6 µg/mL and 218 µg/ml at Ce of 1 and 5 µg/ml were observed by t = 1 h. This corresponds to Ci/Ce ratios of 31.2 and 41.2. Azithromycin values were similar to those from published studies with Ci/Ce ratios of 14.9 (Ce, 1 µg/ml) and 19.5 (Ce, 5 µg/ml) at t = 5 h. The antibacterial activity of BC-3781 remained unaffected by the addition of Survanta™. None of the isolates tested had BC-3781 MICs that increased more than two-fold. Daptomycin MICs against *S. aureus* and *S. pneumoniae* increased by up to ≥160-fold, with increasing surfactant concentrations.

Conclusion: Overall, BC-3781 accumulated in murine macrophages at clinically relevant extracellular concentrations and the antimicrobial potency of BC-3781 was unaffected by lung surfactant. Together with the high tissue penetration into the lung, these data support the investigation of BC-3781 in bacterial pneumonia.

INTRODUCTION

Community-acquired bacterial pneumonia (CABP) is one of the most common infections worldwide in humans (5-10 million cases and 1.1 million hospitalizations in US annually) which causes significant morbidity and mortality (CDC 2009). Despite a number of antibiotics available to treat this infection, new antibacterial agents are needed to overcome resistance development.

BC-3781 is a novel, systemic pleuromutilin (oral and i.v.) with a spectrum of activity consistent with the organisms typically responsible for CABP such as *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Staphylococcus aureus* (MSSA and MRSA), *Legionella pneumophila*, *Chlamydophila pneumoniae* and *Mycoplasma pneumoniae*.[1] BC-3781 also demonstrated good tissue penetration into the lung and favorable efficacy in murine pneumonia models suggesting further development in this indication.[2]

A factor related to good tissue penetration is the intracellular accumulation in phagocytes, which has been discussed extensively with macrolides. Accumulation of drugs within polymorphonuclear cells and partitioning into various intracellular compartments can lead to improved distribution into the tissue surrounding the site of infection.[3] This study investigated the accumulation of BC-3781 in macrophages in comparison with azithromycin.

Previous publications have demonstrated the adverse effect of bovine lung surfactant on daptomycin *in vitro* activity, which translated to inferiority of daptomycin in phase 3 CABP trials.[4,5] This effect appears to be specific for daptomycin due to its specific interaction with the Gram-positive plasma membrane; however, new antibiotics that are under consideration for pneumonia indications are now routinely evaluated for potential interactions with lung surfactant. This study evaluated the potential for an effect of lung surfactant on the potency of BC-3781 in comparison to the known effect on daptomycin.

METHODS

BC-3781 Uptake in Macrophages

J774 mouse macrophages were grown in RPMI 1640 (+10 % inactivated FCS) and plated at a concentration of 2 x 10^6 cells/mL and incubated at 37 °C (5 % CO_2) for 24 h. Adherent cells were incubated with 1 and 5 µg/ml BC-3781 in RPMI for up to 5 hours at 37 °C (5 % CO_2). Azithromycin and penicillin served as positive and negative controls.

After incubation the test medium was aspirated, cells were washed and further disrupted by three freeze-thaw cycles. The intracellular (Ci) and extracellular concentrations (Ce) in cell lysate was determined after protein precipitation with ice-cold acetonitrile in triplicate by LC-MS/MS in ESI positive mode using a 1100 Series high pressure gradient HPLC system (Agilent Technologies) coupled to a triple quadrupol MS (G6410A, Agilent Technologies).

The Ci/Ce ratio was calculated using the assumption that 1 mg of cellular protein (determined by Bradford assay) is equivalent to a cellular volume of 5 µL, as reported for peritoneal macrophages or cultured fibroblasts.[6] Additionally, the intracellular volume was confirmed by the mini cell counter MoxiZ™ (Orflo Technologies, Hailey, USA). Additional samples were taken at t = 0 for determination of Ce and for proof of compound stability during the lysis procedure.

Effect of Lung Surfactant on the BC-3781 *In Vitro* Activity

The MIC of BC-3781 and daptomycin in presence of 15-1000 µg/ml bovine lung surfactant (Survanta™, Abbot) was evaluated by the checkerboard broth microdilution technique according to CLSI M100-S22 using an inoculum of 5x10^5 CFU/ml and the following growth media: CAMHB for *S. aureus* (MSSA and MRSA) and *E. coli*, CAMHB supplemented with 2-5% laked horse blood for *S. pneumoniae*, HTM for *H. influenzae*. For daptomycin the Ca^{2+} concentration was adjusted to 50 mg/ml. The highest concentration of lung surfactant tested was 1000 µg/mL (a 1:25 dilution of Survanta™ corresponding to 4% (v/v)).

RESULTS

BC-3781 Uptake in Macrophages

- The intracellular concentration and accumulation of test compounds within the murine J774 macrophages is shown in Figures 1-2.
- BC-3781 showed approximately 30- to 50-fold accumulation in the macrophages at extracellular concentrations (Ce) of 1 µg/mL and 5 µg/mL after 5 h of incubation.
- The BC-3781 uptake appeared to be a fast process since significant Ci of approximately 35 µg/mL (Ce, 1 µg/mL) and 220 µg/ml (Ce, 5 µg/mL) were reached already at 1 h, corresponding to Ci/Ce ratios of ~30-40.
- The accumulation of azithromycin was, with Ci/Ce ratios of approximately 15-20 at 5 h, in the range of published values and lower than that of BC-3781. Penicillin G, used as an example of an antibiotic not accumulating within macrophages, displayed a Ci below the Ce.

Effect of Lung Surfactant on the BC-3781 *In Vitro* Activity

- Checkerboard experiments showed that bovine lung surfactant (Survanta™) had no effect on the MIC of BC-3781 against the isolates tested (Table 1). None of the eight isolates tested had an increase in BC-3781 MIC that was more than 2-fold at any Survanta™ concentration (Figure 3A).
- In contrast, daptomycin MICs against *S. aureus* and *S. pneumoniae* significantly increased with increasing concentrations of Survanta™ bovine lung surfactant. A >2-fold MIC increase was observed already at the lowest Survanta™ concentration and MICs continued to increase by as much as ≥160-fold with increasing Survanta™ concentrations. *H. influenzae* and *E. coli* were intrinsically resistant to daptomycin and were therefore not evaluated against this agent (Table 1 and Figure 3B).

Figure 1. Intracellular concentration (Ci) and Ci/Ce ratios of BC-3781 and comparators in macrophages at the extracellular drug concentration (Ce) of A) 1 µg/ml and B) 5 µg/ml

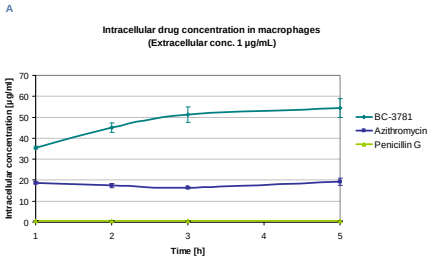


Figure 2. Ci/Ce ratios of BC-3781 and comparators in macrophages over time

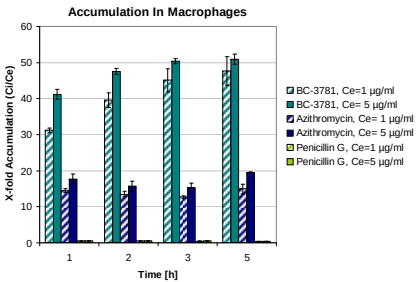
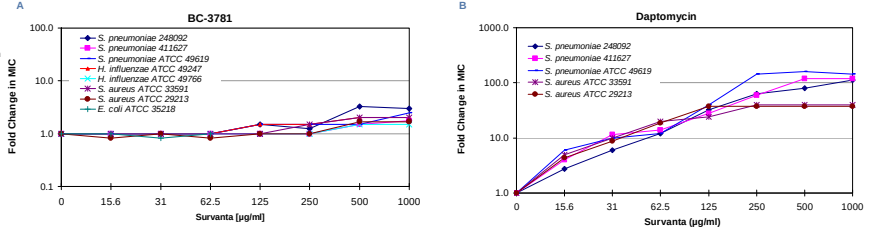


Table 1. MIC [µg/ml] of BC-3781 and daptomycin in presence of increasing concentrations of the lung surfactant Survanta™ (n, number of experiments)

	Survanta™ Concentration		MIC [µg/ml]							
			S. pneumoniae	*S. pneumoniae*	*S. pneumoniae*	*H. influenzae*	*H. influenzae*	*S. aureus*	*S. aureus*	*E. coli*
Antibiotic	[µg/mL]	[%] (v/v)	248092 (n = 4)	411627 (n = 4)	ATCC 49619 (n = 2)	ATCC 49247 (n = 2)	ATCC 49766 (n = 2)	ATCC 33591 (n = 2)	ATCC 29213 (n = 3)	ATCC 35218 (n = 2)
BC-3781	0	0	0.06 - 0.12	0.03 - 0.06	0.03 - 0.06	0.5 - 1	1-2	0.06 - 0.12	0.12	16
	15	0.06	0.06 - 0.12	0.03 - 0.06	0.03 - 0.06	0.5 - 1	1-2	0.06 - 0.12	0.06 - 0.12	16
	31	0.12	0.06 - 0.12	0.03 - 0.06	0.03 - 0.06	0.5 - 1	1-2	0.06 - 0.12	0.12	8-16
	62.5	0.25	0.06 - 0.12	0.03 - 0.06	0.03 - 0.06	0.5 - 1	1-2	0.06 - 0.12	0.06 - 0.12	16
	125	0.5	0.06 - 0.25	0.03 - 0.06	0.06	0.5, 2	1-2	0.06 - 0.12	0.12	16
	250	1	0.06 - 0.12	0.03 - 0.06	0.06	0.5, 2	1-2	0.12	0.12	16
	500	2	ND	ND	ND	ND	ND	0.12 - 0.25	0.12 - 0.25	16
	1000	4	ND	ND	ND	ND	ND	0.12 - 0.25	0.12 - 0.25	16
Daptomycin	0	0	0.12 - 0.25	0.06 - 0.25	0.06 - 0.125	>16	>16	0.5 - 1	0.25 - 1	>16
	15	0.06	0.25 - 0.5	0.25 - 0.5	0.25 - 0.5	>16	>16	2	1 - 2	>16
	31	0.12	0.5 - 1	0.25 - 2	0.25, 1	>16	>16	4	2 - 4	>16
	62.5	0.25	1 - 2	0.5 - 2	0.5 - 1	>16	>16	8	8	>16
	125	0.5	1 - 8	1, 4 - 8	1, 4	>16	>16	8 - 16	16	>16
	250	1	4 - 16	1, 4 - 8	2, 16	>16	>16	16	16	>16
	500	2	8 - 16	2, 16	4, 16	>16	>16	16	16	>16
	1000	4	16	2, 16	2, 16	>16	>16	16	16	>16

ND, not determined because of high turbidity of growth medium

Figure 3. Average fold change in MIC values of BC-3781 (A) and daptomycin (B) with increasing amounts of Survanta™ lung surfactant



CONCLUSIONS

- BC-3781 displayed a rapid 30- to 50-fold accumulation into murine macrophages dependent on the time of incubation at clinically relevant extracellular concentrations.
- The antimicrobial potency of BC-3781 for organisms commonly causing CABP such as *S. aureus*, *S. pneumoniae* and *H. influenzae* was unaffected by lung surfactant.
- Together with the high tissue penetration into the lung and the overall potent antibacterial profile including atypicals such as *C. pneumoniae*, *M. pneumoniae* and *L. pneumophila*, these data support the further investigation of BC-3781 in bacterial pneumonia.

ACKNOWLEDGMENTS

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