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Efficacy of BC-3781 in Murine Pneumonia Models

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ABSTRACT

Background: BC-3781 is a novel pleuromutilin antimicrobial agent currently in clinical development for intravenous and/or oral treatment of acute bacterial skin and skin structure infections (ABSSSI) and community-acquired bacterial pneumonia (CABP). BC-3781 exhibits excellent antimicrobial activity against a range of relevant bacteria frequently identified in CABP, including *Streptococcus pneumoniae* (SP), *Haemophilus influenzae* (HI) and methicillin-resistant *Staphylococcus aureus* (MRSA). CABP is a leading cause of morbidity and mortality. Current treatments are often unsatisfactory and novel antibacterials with improved efficacy against severe CABP infections are urgently needed.

Methods: Murine pulmonary infections caused by SP, HI and MRSA were established in female BALB/c mice. To establish a necrotizing MRSA pneumonia in mice, neutropenia was induced by 2 injections of cyclophosphamide (150 and 100 mg/kg) 4 and 1 day before infection. The mice were inoculated by placing 50 µl onto tip of nares. Inoculum size of SP, HI, and MRSA was $3x10^6$, $2.3x10^6$ and $1.5x10^7$ CFU/mouse, respectively. Therapy was initiated 2 h p.i. for HI and MRSA pneumonia with a single dose and continued for two days with a bid dosing regimen. SP induced pneumonia was treated with a single dose 16 h p.i.; dissection at 24 h p.a.. The bacterial burden in pulmonary tissues was determined using standard plating techniques. An Emax dose response model was used to obtain the bacteriostatic dose levels and the maximum killing potency of all tested compounds. Additionally, plasma and ELF concentrations of BC-3781 were investigated.

Results: BC-3781 showed excellent efficacy against SP, HI and MRSA with a reduction of 3 log10 CFU/lung being achieved with doses of 150-160 mg/kg BC 3781 s.c.. Compared to the standard of care antibiotics, BC-3781 showed excellent *in vivo* activity in lung infections.

Conclusions: BC-3781 demonstrated excellent efficacy in murine pulmonary infections caused by SP, HI or MRSA. In models employed, the BC-3781 efficacy was superior to the standard of care antibiotics.

INTRODUCTION

Pneumonia is a leading cause of morbidity and mortality worldwide and the sixth most common cause of death in the USA. [1]

Nabriva's compound BC-3781 is a novel representative of pleuromutilins, a new class of antibiotics for human use demonstrating excellent potency against resistant bacterial pathogens, amongst them methicillin-reistant *Staphylococcus aureus* (MRSA), including its community-acquired strains (CA MRSA), and multi-drug resistant pneumococci. [2,3] BC-3781 is currently in a Phase II clinical trial for the indication of acute bacterial skin and skin structure infections (ABSSSI).

In *in vitro* and *in vivo* studies BC-3781 exhibited time-dependent killing, showed bacteriocidal (*Streptococcus pneumoniae* and *Haemophilus influenzae*) and bacteriostatic (*S. aureus*) activity and produced a modest post antibiotic effect (PAE). In general, the plasma concentration-time curve of intravenously administered BC-3781 in humans and animals showed a multi-phasic decline. Following the end of infusion (maximum plasma concentration, C_{max}), there was a rapid distribution phase over 0.5 h followed by an extended elimination phase followed by an terminal phase with a mean half-life of 8.6 h to 11.7 h after single dosing. [4]

The overall very good tissue penetration properties observed in a quantitative whole body autoradiography (QWBA) study in rats after intravenous application of BC-3781, substatiated by both, high exposures levels of BC-3781 in the epithelial lining fluid (ELF) of mice and excellent murine bronchiopneumonia *in vivo* efficacy data, potentially qualifies BC-3781 for the indication of bacterial respiratory tract infections (RTI).

MATERIALS & METHODS

Animals: Female BALB/c mice weighting 20-25 grams were allowed to acclimate approximately one week and were utilized throughout the experiments.

Minimal inhibitory concentrations (MIC): MIC were determined by CLSI broth microdilution method. [5]

***In vivo* efficacy:** The *in vivo* antibacterial activity of BC-3781 against severe bacterial lung infections was determined using murine bronchopneumonia infection models. Infections caused by *S. pneumoniae* and *H. influenzae* were established in immuno-competent mice. To induce a life threatening necrotizing MRSA pneumonia mice were rendered neutropenic (cyclophosphamide) four days and one day before infection. The mice were inoculated by placing 50 µl onto tip of nares. Innoculum size for *S. pneumoniae*, *H. influenzae*, and MRSA was $3x10^6$, $2.3x10^6$ and $1.5x10^7$ CFU/mouse, respectively. Therapy was initiated 2 h p.i. for *H. influenzae* and MRSA pneumonia with single doses of test drugs and continued for two days with a *bid* dosing regimen. *S. pneumoniae* induced pneumonia was treated with a single dose 16 h p.i.; dissection at 24 h p.a.. The bacterial burden in pulmonary tissues was determined using standard plating techniques. An E_{max} dose response model was used to obtain the bacteriostatic doses, the dose required to reduce 1 log_{10} CFU/lung ($-1log_{10}$ dose) levels, and the maximum killing potency (E_{max}) of all tested compounds.

Pharmacokinetic studies: Blood and bronchioalveolar lavage (BAL) samples were collected simultaneously after dosing of BC-3781 to non-infected animals.

Plasma and BAL were assayed for urea to allow for determination of drug levels in the ELF. Analysis of BC-3781 plasma and BAL concentrations was done with HPLC-MS/MS.

RESULTS

Following s.c. administration of BC-3781 and comparator agents (moxifloxacin, azithromycin, linezolid and vancomycin) the activity was confirmed in all three models.

- In *S. pneumonia* respiratory tract infection model BC-3781 showed superior efficacy to all comparators (Figure 1).
- BC-3781 showed greater efficacy than linezolid and similar maximum killing potency compared to moxifloxacin (Table 1).

Table 1. The MICs, E_{max}, and static and $-1log_{10}$ doses obtained in the tested murine pneumonia models

Strain	Compound	MIC [µg/mL]	E_{max} log_{10} CFU/Lung	Static Dose [mg/kg/day]	$-1log_{10}$ Dose [mg/kg/day]	No of Doses
S. pneumoniae ATCC 6303	BC-3781	0.06	-2.66	5.55	12.6	1
	Moxifloxacin	0.12	-2.84	7.41	28.3	1
	Linezolid	0.5	-1.33	8.35	77.4	1
H. influenzae clinical isolate	BC-3781	1	-4.95	54.9	76.1	3
	Azithromycin	2	-4.57	16.1	27.2	3
S. aureus MRSA clinical isolate	BC-3781	0.12	-3.75	44.1	53.7	5
	Linezolid	2	-1.47	80.5	108	5
	Vancomycin	0.5	-5.18	177	291	5
S. aureus CA-MRSA USA300	BC-3781	0.12	-5.49	38.3	46.2	5
	Linezolid	2	-4.80	74.9	85.4	5
	Vancomycin	0.5	-5.19	120	133	5

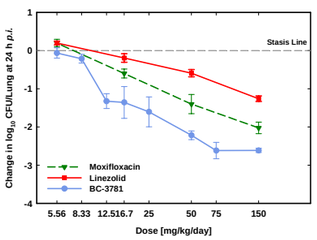
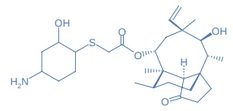


Figure 1: Dose-response relationships for subcutaneously dosed BC-3781, moxifloxacin and linezolid against *S. pneumoniae* ATCC 6303 in the lungs of non-neutropenic mice

BC-3781



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- BC-3781 and azithromycin showed good activity against murine bronchopneumonia caused by *H. influenzae*. The bacteriostatic dose of BC-3781 of 54.9 mg/kg/day is only slightly higher compared to the static dose observed in the MRSA pneumonia (Table 1). The maximum killing potency of BC-3781 and azithromycin after 47 h p.i. was similar.
- The bactiostatic doses of BC-3781 were much lower than for any of the comparators tested in the MRSA pneumonia model (Table 1). BC-3781 showed the lowest effective dose (survival breakpoint) when treating life threatening necrotizing MRSA pneumonia (Figures 2 and 3).
- The bacteriostatic dose of BC-3781 determined in the MRSA pneumonia is about 2-fold lower compared to the static dose level obtained in the thigh model. [6] The comparator linezolid showed similar static doses in lungs in thighs. [7]
- BC-3781 showed excellent penetration (~200%) into ELF (Figure 4).

Figure 2: Dose-response effects of subcutaneously dosed BC-3781, vancomycin and linezolid on the density of MRSA in the lungs of neutropenic mice

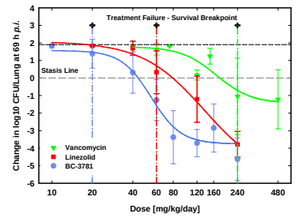


Figure 3: Dose-response effects of subcutaneously dosed BC-3781, vancomycin and linezolid on the density of CA-MRSA(USA300) in the lungs of neutropenic mice

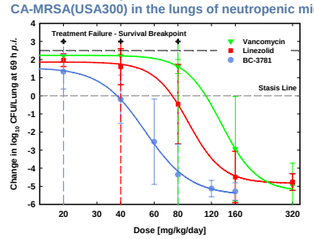
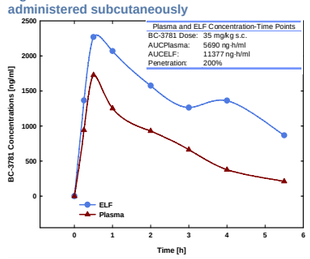


Figure 4: Penetration of BC-3781 into murine ELF (blue) at dose of 35 mg/kg administered subcutaneously



CONCLUSIONS

- BC-3781 exhibited excellent efficacy in respiratory tract infections caused by *S. pneumoniae*, *H. influenzae*, and MRSA, including CA-MRSA.
- High exposure levels of BC-3781 in the ELF and its broad-spectrum activity against respiratory pathogens are strongly supporting its potential use in the treatment of bacterial respiratory tract infections.

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