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Pre-clinical Efficacy of BC-3781 in Thigh and Bacteremia Infections Caused by Staphylococci

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ABSTRACT

Background: The pleuromutilin BC-3781 is a new antimicrobial agent, which is in clinical development for intravenous and 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 methicillin sensitive and resistant *Staphylococcus aureus* (MSSA and MRSA) strains.
Methods: Murine peritonitis models inducing lethal bacteremia caused by MSSA ($3.8x10^7$ CFU/mouse) or MRSA ($7x10^6$ CFU/mouse) strains were established in immuno-competent female mice. The antibiotics (BC-3781, linezolid and vancomycin) were dosed s.c. 1 h and 4 h p.i.. The ED50 and 95% confidence limits were determined by probit analysis. Further the efficacy of BC-3781 was compared to that of linezolid and vancomycin in a thigh infection model. The infection caused by the MSSA strain was established in neutropenic mice, the MRSA infection was established in immuno-competent mice. The twice daily antimicrobial treatment (50 mg/kg bid s.c.) was initiated 1 h p.i. for two consecutive days. At start of the therapy CFU/thigh reached $2.4x10^6$ (MSSA) and $5.8x10^6$ (MRSA). At the end of the treatment period the viable cell counts and the reduction of CFU/thigh after 48 h compared to the CFU/thigh at the onset of treatment were calculated.
Results: In bacteremia models BC-3781 showed superior efficacy when compared to linezolid and vancomycin, with ED_{50} values of 1.77 mg/kg/day (MSSA) and 0.23 mg/kg/day (MRSA). The *in vivo* activity of BC-3781 observed in the MRSA thigh model after a 48 h treatment was comparable with that of s.c. administered vancomycin and linezolid. In neutropenic mice infected with MSSA strain, 100 mg/kg/day of linezolid showed static CFU levels after 48 h, whereas BC-3781 and vancomycin achieved a CFU reduction of more than 2.5 log_{10}.
Conclusions: BC-3781 demonstrated excellent efficacy in murine bacteremia and thigh infection caused by MSSA and MRSA.

INTRODUCTION

Staphylococcus aureus is a leading cause of bacteremia often associated with serious complications, including endocarditis, osteomyelitis, and prosthetic-joint infections. The extensive use of vancomycin, the current standard treatment for staphylococcal bloodstream infections, is likely linked to the emergence of *S. aureus* strains with reduced susceptibility to vancomycin (RVS). In particular, strains of vancomycin-intermediate *S. aureus* (VISA) and heteroresistant *S. aureus* (hVISA) have been associated with vancomycin treatment failures. Nabriva's compound BC-3781 is a novel representative of pleuromutilins, a new class of antibiotics for human use demonstrating excellent potency against gram-positive pathogens, including methicillin-resistant *S. aureus* (MRSA), fastidious gram-negative bacteria, including *Haemophilus* spp., *Moraxella* spp. and species of atypical pathogens. [1,2,3] BC-3781 is currently in a Phase II clinical trial in acute bacterial skin and skin structure infections (ABSSSI) being the first systemically available pleuromutilin antibiotic administered to patients.

In *in vitro* and *in vivo* studies, BC-3781 exhibited time-dependent killing, showed predominately bacteriostatic properties against *S. aureus* (bactericidal against *Streptococcus pneumoniae* and *Haemophilus influenzae*) and produced a modest post antibiotic effect. The 24 h AUC/MIC ratio followed by the $T_{>MIC}$ were identified as parameters correlating best with efficacy. The *in vivo* breakpoint associated with efficacy was determined against *S. aureus* and *S. pneumoniae* based on the murine thigh infection model. A mean AUC/MIC ratio of 57.5 corresponding to a *f*AUC/MIC target for efficacy of 11.5 has been determined to be appropriate for the treatment of acute bacterial skin and skin structure infections (ABSSSI). [4].
The plasma concentration-time curve of intravenously administered BC-3781 in humans shows a multi-phasic decline. Following the end of infusion (maximum plasma concentration, C_{max}), there is a rapid distributional 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. [6,7]
The observed large volume of distribution at steady state, 2- to 5-fold greater than total body water of 42 l, indicated a low affinity to plasma proteins as well as good and rapid distribution into tissues and organs, which was substantiated by quantitative whole body autoradiography (QWBA). [8] The excellent activity of BC-3781 compared to linezolid and vancomycin in the above described thigh infection model reflect these favorable PK/PD characteristics.
To test the efficacy of BC-3781 against blood stream infections a murine peritonitis models was used which leads if untreated to lethal bacteremia within 24 h. Again, BC-3781 showed superior activity compared to linezolid and vancomycin suggesting a potential use in patients suffering from staphylococcal blood stream infections.

MATERIALS & METHODS

Bacterial isolates: Minimal inhibitory concentrations (MIC): MICs were determined by CLSI broth microdilution method. [5]
***In vivo* efficacy:** The *in vivo* antibacterial activity of BC-3781 against bloodstream infections was determined in a murine septicemia infection model in immuno-competent mice. MSSA strains B9 (ATCC 49951) and MRSA strain B29 (clinical isolate) were used as infectious agents. The total daily dose required for survival of 50% of mice at 96 h post infection (ED_{50}) and 95% confidence limits were determined by the binary probit analysis. The inoculum ranged between $7x10^6$ and $6x10^7$ CFU/mouse.
The efficacy of BC-3781 against systemic organ infections was evaluated in a thigh infection model using immuno-competent mice infected with *S. aureus* B29 (MRSA) and neutropenic mice infected with *S. aureus* B399 (MSSA; ATCC 13709). Differences in viable counts between the controls, drug-untreated and drug-treated groups, were analyzed by Dunnett's multiple-comparison procedure, while the Bonferroni's t-test was used to examine statistical differences between BC-3781 and the standard-of-care antibiotics vancomycin and linezolid. Subcutaneous administration of fixed doses of 100 mg/kg/day (50 mg/kg *bid*) for two consecutive days of BC-3781, vancomycin and linezolid allowed the side-by-side comparison of reduction of viable cell counts in the MSSA thigh infection model in neutropenic mice.

RESULTS

- In septicemic infections in mice induced by MSSA the efficacy of parenterally administered BC 3781 was superior to linezolid and equivalent to vancomycin (Figure 1).
- The efficacy of BC-3781 against MRSA induced peritonitis was superior to vancomycin and linezolid (Figure 2).

Figure 1. *In vivo* activity of BC 3781, linezolid and vancomycin in *S. aureus* B9 (MSSA, ATCC 49951) sepsis model in immuno-competent mice

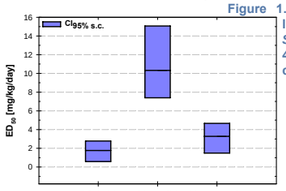
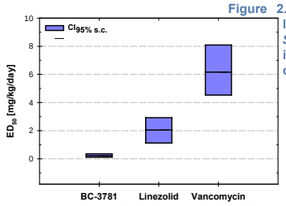


Figure 2. *In vivo* activity of BC 3781, linezolid and vancomycin in *S. aureus* B29 (MRSA, clinical isolate) sepsis model in immuno-competent mice



- BC-3781 and vancomycin showed significant reduction of viable cell counts in infected thighs (Table 1 and Figure 3).
- 100 mg/kg/day of linezolid produce a net static effect against S. aureus ATCC 13701 in neutropenic mice (Table 1 and Figure 3) as reported previously. [9]

Table 1. Efficacy of BC-3781 and reference antibiotics against murine thigh infection caused by *S. aureus* (MSSA, ATCC 13709) in neutropenic mice

Compound	Dose [mg/kg/day] bid	Route	MIC [µg/mL]	Viable Counts $logB_{10B}$ CFU/Thigh Mean ± SD	Δlog_{10} CFU/Thigh
Pretreated (*t* = 0 h)	-	-	-	6.38 ± 0.10	±0.00
Untreated (*t* = 48 h)	-	-	-	9.47 ± 0.18 [a]	+3.09
BC-3781	100	s.c.	0.05	3.72 ± 1.33 [a]	-2.66
Vancomycin	100	s.c.	1	2.95 ± 0.87 [a]	-3.44
Linezolid	100	s.c.	2	6.42 ± 2.48 [a]	+0.04

[a] P < 0.05 compared with early control (Dunnett´s method)

Figure 3. Therapeutic efficacy of BC-3781 and reference antibiotics against murine thigh infection caused by *S. aureus* (MSSA, ATCC 13709) in neutropenic mice

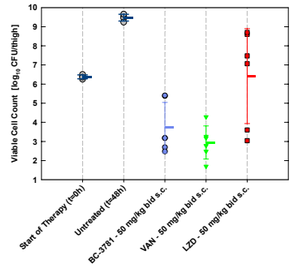
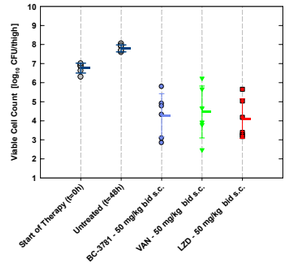


Table 2. Efficacy of BC-3781 and reference antibiotics against murine thigh infection caused by *S. aureus* (MRSA, clinical isolate) in non-neutropenic mice

Compound	Dose [mg/kg/day] bid	Route	MIC [µg/mL]	Viable Counts log_{10} CFU/Thigh Mean ± SD	Δlog_{10} CFU/Thigh
Pretreated (t = 0 h)	-	-	-	6.76 ± 0.26	±0.00
Untreated (t = 48 h)	-	-	-	7.80 ± 0.19	+1.04
BC-3781	100	s.c.	0.12	4.29 ± 1.13 [a]	-2.48
Vancomycin	100	s.c.	1	4.46 ± 1.36 [a]	-2.31
Linezolid	100	s.c.	2	4.12 ± 1.04 [a]	-2.65

[a] P < 0.05 compared with early control (Dunnett´s method)

Figure 4. Therapeutic efficacy of BC-3781 and reference antibiotics against murine thigh infection caused by *S. aureus* B29 (MRSA) in immuno-competent mice



CONCLUSIONS

- BC-3781 demonstrated excellent efficacy, being equivalent to vancomycin and superior to linezolid, against murine bacteremia infections caused by MSSA and MRSA thus representing a potential valuable new treatment option.
- Against staphylococcal induced thigh infections BC-3781 demonstrated a significant reduction of viable bacteria at site of infection, with efficacy equivalent to vancomycin and equivalent (in MRSA) or superior (in MSSA) efficacy when compared to linezolid.
- The immune status of mice did not influence BC-3781 efficacy.

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