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In Vivo Pharmacodynamic Activity of BC-3781

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ABSTRACT

Background: BC-3781 is an antimicrobial agent of the pleuromutilin class inhibiting the prokaryotic protein synthesis. BC-3781 is undergoing clinical development for intravenous and oral treatments of acute bacterial skin and skin structure infections (ABSSSI) and community-acquired bacterial pneumonia (CABP). We examined the pharmacodynamics of BC-3781 against clinical isolates of *Staphylococcus aureus* (including methicillin-resistant *S. aureus*) and *Streptococcus pneumoniae* (including penicillin-resistant *S. pneumoniae*) in neutropenic murine thigh and lung infection models.
Methods: Mice were treated with total doses of 5 to 160 mg/kg given as single doses or as fractionated doses given 2, 4 or 8 times per day to determine which PK/PD index is most relevant for efficacy. To investigate *in vivo* killing and post antibiotic effects (PAEs), single doses of 10, 20 and 40 mg/kg were administered subcutaneously. At the start of therapy mice had 10^5 to 10^7 CFU/thigh or lung of either *S. aureus* or *S. pneumoniae*. The 12 hour dose required for a net bacteriostatic effect was determined using the E_{max} dose-response model. Plasma protein binding was determined by equilibrium dialysis.
Results: BC-3781 exhibited time-dependent killing with moderate post-antibiotic effects (PAEs) of 1-3 hours. The 24 h AUC/MIC followed by $T_{>MIC}$ were identified as the PK/PD indices most important for efficacy. The drug appeared to have 3- to 6-fold higher potency in the lung than the thigh. The magnitude of the 24-h AUC/MIC of total drug required for a static effect with the various strains of *S. pneumoniae* and *S. aureus* ranged from 9.92-82.5. This would correspond to free drug values ranging from 1.98-16.5 based on the *in vitro* plasma protein binding in mice of 80%.
Conclusions: The mean bacteriostatic free drug 24 h AUC/MIC ratio of 11.5 covers both staphylococci and pneumococci. This free drug AUC/MIC ratio provides the basis for a target attainment analysis in ABSSSI and CABP patients' population.

INTRODUCTION

The below mentioned studies were performed to characterize the *in vivo* pharmacodynamic properties of BC-3781, a novel pleuromutilin antibiotic, currently in clinical Phase II testing for the indication acute bacterial skin and skin structure infections (ABSSSI): (I) Influence of dosing regimen on the *in vivo* efficacy of BC-3781 determined in a murine thigh infection model in neutropenic mice. (II) Use of a thigh and pneumonia infection model to identify the PK/PD indices (peak concentration, area under the concentration-time curve, duration of time plasma levels exceeding MIC) associated best with efficacy.

MATERIALS & METHODS

Pharmacokinetics: The plasma PK of BC-3781 in thigh infected neutropenic mice was determined by HPLC-MS/MS.
***In Vivo* Efficacy:** The neutropenic murine thigh infection model and the lung infection model were used to determine the *in vivo* efficacy of subcutaneously dosed BC-3781. In these well-established models, chemically induced neutropenia was produced by two injections of cyclophosphamide, 150 mg/kg four days prior to infection and 100 mg/kg on the day before infection.

The challenging CFU of five *S. pneumoniae* and eight *S. aureus* strains inducing experimental infection was in the range of $10^{5\text{-}7}$ CFU/mL. The relationship between 24 h AUC/MIC and the reduction in viable cell counts in thighs and lungs was determined using an inhibitory sigmoid E_{max} analysis.

RESULTS

Table 1. Pharmacokinetic properties of single-dose BC-3781 and linezolid (s.c.) in neutropenic mice infected in the thighs with *S. aureus*

Compound	Dose [mg/kg]	C_{max} [µg/mL]	$AUC_{0\text{-}24\ h}$ [µg·h/mL]	$AUC_{0\text{-}12\ h}$ [µg·h/mL]	Protein binding [%]
BC-3781	20	1.28	2.39	2.35	80
	80	1.93	8.18	7.90	80

Parameters Correlating with Efficacy: To determine which PK/PD index correlated best with efficacy of BC-3781 the number of bacteria in the thigh at the end of 24 h of therapy were related with: (I) the C_{max}/MIC ratio, (II) the 24 h AUC/MIC ratio, and (III) the percentage of the dosing interval that plasma levels exceed the MIC for each of the dosage regimens studied ($T_{>MIC}$).
Free drug concentrations were used to calculate the magnitude of the various PK/PD indices. Figure 1 and Figure 2 depict the coefficient of determination observed for the relationship between efficacy and each PK/PD index for *S. pneumoniae* and *S. aureus*, respectively. The best correlations were observed with the 24 h AUC/MIC followed by the $T_{>MIC}$ ratios.

Figure 1. Relationship of different PK/PD indices on the antimicrobial activity of BC-3781 against *S. pneumoniae* ATCC 10813 in the thighs of neutropenic mice

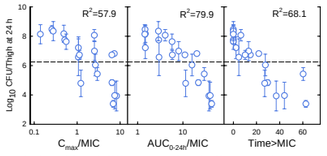
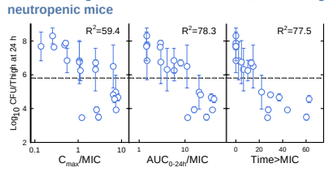


Figure 2. Relationship of different PK/PD indices on the antimicrobial activity of BC-3781 against *S. aureus* ATCC 25923 in the thighs of neutropenic mice



The effect of single doses of 10, 20 and 40 mg/kg of BC-3781 on the *in vivo* killing and re-growth of *S. pneumoniae* ATCC 10813 is shown in Figure 3. Each point represents the mean of three mice. The rate of killing of the strain of *S. pneumoniae* was not increased by higher drug concentrations. However, re-growth of *S. pneumoniae* began at 4 h at 10 mg/kg and at 6 h with 40 mg/kg; re-growth at 20 mg/kg was intermediate between the other two doses. This suggests that the *in vivo* post-antibiotic effect (PAE) with *S. pneumoniae* was around 3.0 to 3.5 h for BC-3781.

The effect of single doses of 10, 20 and 40 mg/kg dose of BC-3781 on the *in vivo* killing and re-growth of *S. aureus* ATCC 25923 is shown in Figure 4. Each point represents the mean of three mice. The rate of killing was not enhanced by higher serum concentrations.

Furthermore, re-growth with *S. aureus* began at 2 h at 10 mg/kg and at 4 h with 40 mg/kg; re-growth at 20 mg/kg was again intermediate between the other two doses. This suggests that the *in vivo* post-antibiotic effect (PAE) with *S. aureus* corresponds to 1.0 and 1.5 h.

Figure 3. Effect of single doses of BC-3781 on time course of antimicrobial activity with *S. pneumoniae* ATCC 10813 in the thighs of neutropenic mice

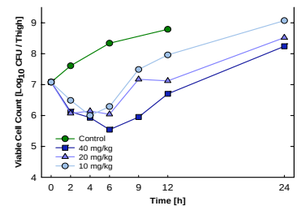
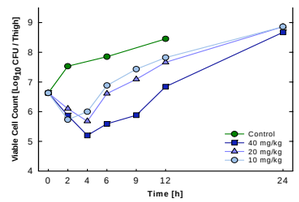
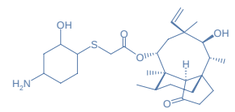


Figure 4. Effect of single doses of BC-3781 on time course of antimicrobial activity with *S. aureus* ATCC 25923 in the thighs of neutropenic mice



BC-3781



***In vivo* Breakpoints Associated with Efficacy:** The *in vivo* pharmacodynamic activity of BC-3781 was evaluated against *S. aureus* as predominant ABSSSI pathogen. The activity of q12 dosing regimens of BC-3781 was investigated in two PK/PD murine thigh infection studies with similar set up. The *S. aureus* group included two MSSA and six MRSA strains, including hospital-associated and community-acquired strains. The pharmacodynamics of BC-3781 was similar for all *S. aureus* strains showing a good *in vitro* - *in vivo* correlation (MIC - static dose relationship). The total AUC/MIC values for each dose were calculated based on PK parameters of infected mice and normalized over the free fraction, as determined by *in vitro* experiments using equilibrium dialysis method. Accordingly, a value of 20% unbound BC-3781 was used for calculations of 24 h *f*AUC/MIC. The individual efficacy at corresponding 24 h *f*AUC/MIC ratios of eight *S. aureus* strains out of two studies and the sigmoid curve fit of mean and $CI_{95\%}$ are depicted in Figure 5. The 24 h *f*AUC/MIC necessary to produce a net static effect against *S. aureus* was 11.5 with a 95% confidence interval band of 9.69 to 13.4. The mean 24 h *f*AUC/MIC necessary to produce a net static effect against *S. pneumoniae*, depicted in Figure 6, was with 3.59 with a 95% confidence interval band of 2.45 to 4.60.

Figure 5. Inhibitory E_{max} model and 95% confidence interval of 24h *f*AUC/MIC as a function of change in CFU/thigh for *S. aureus* isolates

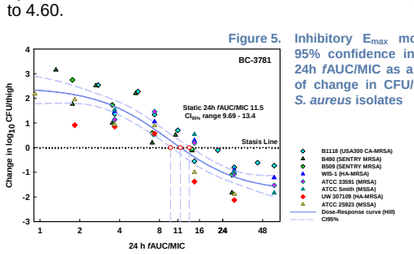
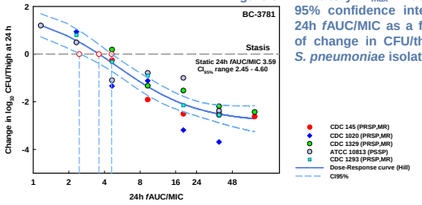
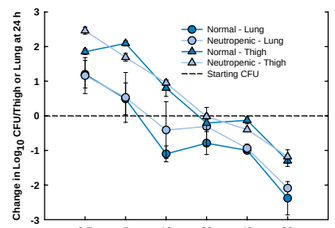


Figure 6. Inhibitory E_{max} model and 95% confidence interval of 24h *f*AUC/MIC as a function of change in CFU/thigh for *S. pneumoniae* isolates



Impact of Neutrophils and Site of Infection on Activity of BC-3781: *S. pneumoniae* ATCC 10813 is capable of infecting the thighs and lungs of both normal and neutropenic mice. In these experiments, the lungs and the thighs were infected in the same mice. The dose-response relationship determined in the thighs and lungs of both, neutropenic and normal mice, only showed a marginal effect in enhancing activity of BC-3781 in immuno-competent mice (Figure 7).

Figure 7. Dose-response relationships for 12-hourly dosing of BC-3781 (s.c.) against *S. pneumoniae* ATCC 10813 in the thighs and lungs of normal and neutropenic mice



CONCLUSIONS

- The 24 h AUC/MIC and the $T_{>MIC}$ were the PK/PD indices most important for the efficacy of BC-3781.
- BC 3781 appears to exhibit time-dependent killing but also produces modest *in vivo* PAEs.
- The mean 24 h *f*AUC/MIC necessary to produce a net static effect against *S. aureus* was 11.5.
- BC 3781 was more potent (about 3-fold) in the lung compared to the thigh in both neutropenic and normal mice.
- The presence of white blood cells had only a marginal effect in enhancing the activity of BC-3781.

SELECTED REFERENCES

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- W. A. Craig. Pharmacodynamics of antimicrobials: General concepts and applications. In: Nightingale CH, Murakawa T, Ambrose PG, Eds., Antimicrobial Pharmacodynamics in Therory and Clinical Practice, 2nd edition (2007). New York, NY

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