P907

ECCMID 2010

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 in clinical development for intravenous and oral treatment of skin and skin structure infections (SSSI) and community-acquired pneumonia (CAP). 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 a neutropenic murine thigh and lung infection model.

Methods: Animals were treated either with single doses of 5 to 160 mg/kg or alternatively fractionated doses of 2, 4 or 8 applications per day were given to determine the PK/PD index most relevant for efficacy. To investigate *in vivo* killing and post-antibiotic effects, single doses of 10, 20 and 40 mg/kg were administered subcutaneously. At start of therapy CFU/thigh or lung of either SA or SP in infected mice varied from 10^5 to 10^7. The 12 hour dose required for a net bacteriostatic effect was determined using the E_{max} dose-response model. Plasma protein binding was determined using equilibrium dialysis.

Results: BC-3781 exhibited time-dependent killing with moderate post-antibiotic effects (PAE) of about 3.5 hours for *S. pneumoniae* and around 1.5 hours for *S. aureus*. The 24 h AUC/MIC and $T_{>MIC}$ were identified as the PK/PD indices most important for efficacy. There was only a slightly enhanced activity of the drug in the presence of white blood cells. The drug appeared to have 3- to 6-fold higher potency in the lung than the thigh. The *in vitro* plasma protein binding in mice was 80%.

Conclusions: The bacteriostatic free 24 h *f*AUC/MIC ratio required for a selection of clinical isolates of *S. aureus* and *S. pneumoniae* strains ranged from 8.04 to 16.50. This range provides the basis for the 24 h *f*AUC/MIC target ratio required for efficacy in SSSI and CAP patients.

Introduction

Following studies were performed to characterize the *in vivo* pharmacodynamic properties of BC-3781, a novel pleuromutilin antibiotic. The influence of dosing regimen on the *in vivo* efficacy of BC-3781 was determined in a murine thigh infection model in neutropenic mice. Further, the pre-clinical infection model was used to identify the PK/PD indices (peak concentration, area under the concentration-time curve, the duration of time plasma levels exceed the MIC) associated with efficacy.

Methods

Study Organisms and MICs to BC-3781: MICs were determined in MHB by standard CLSI microdilution techniques. MHB was supplemented with 3 % lysed horse blood for MIC determination with *S. pneumoniae*.

Pharmacokinetics: The plasma PK of BC-3781 in thigh infected neutropenic mice were determined by HPLC/MS/MS.

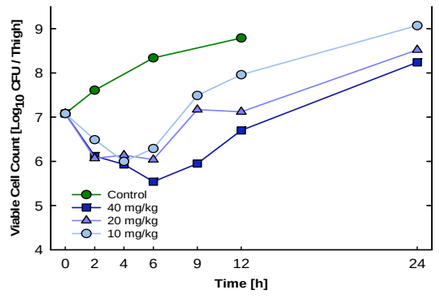
***In Vivo* Efficacy:** The neutropenic murine thigh infection model and the lung infection model were used to address the *in vivo* efficacy of subcutaneously dosed BC-3781. In this well-established model, chemically induced neutropenia was produced by two injections of cyclophosphamide, 150 mg/kg four 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^{5-7} cfu/ml. The relationship between 24 h AUC/MIC and the reduction in viable cell counts in thighs and lungs was determined by using an inhibitory sigmoid E_{max} analysis.

Results

The plasma protein binding determined by equilibrium dialysis at concentrations of 1 and 3 µg/ml was reported to be 80 % in the mouse.

The effect of single doses of 10, 20 and 40 mg/kg of BC-3781 on the *in vivo* killing and regrowth of *S. pneumoniae* ATCC 10813 is shown in Figure 1. Each point represents the mean of three mice. Free drug serum concentrations of BC-3781 were above the MIC for this organism for 0.5 hours at 10 mg/kg and for 3 h at 40 mg/kg. The rate of killing of the strain of *S. pneumoniae* was not increased by higher drug concentrations. However, regrowth of *S. pneumoniae* began at 4 h at 10 mg/kg and at 6 h with 40 mg/kg; regrowth at 20 mg/kg was intermediate between the other two doses. This suggests that the *in vivo* postantibiotic effect (PAE) with *S. pneumoniae* was around 3.0 to 3.5 h for BC-3781.

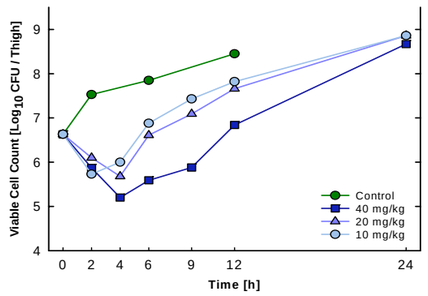
Figure 1. Effect of single doses of BC-3781 on time course of antimicrobial activity with *Streptococcus pneumoniae* ATCC 10813 in the thighs of neutropenic mice



The effect of single doses of 10, 20 and 40 mg/kg dose of BC-3781 on the *in vivo* killing and regrowth of *S. aureus* ATCC 25923 is shown in Figure 2. Each point represents the mean of three mice. The rate of killing was not enhanced by higher serum concentrations. Free drug serum concentrations of BC-3781 were above the MIC for this organism for 0.5 hours at 10 mg/kg and for 3 h at 40 mg/kg.

Furthermore, regrowth with *S. aureus* began at 2 h at 10 mg/kg and at 4 h with 40 mg/kg; regrowth at 20 mg/kg was again intermediate between the other two doses. This suggests that the *in vivo* postantibiotic effect (PAE) with *S. aureus* was only 1.0 and 1.5 h.

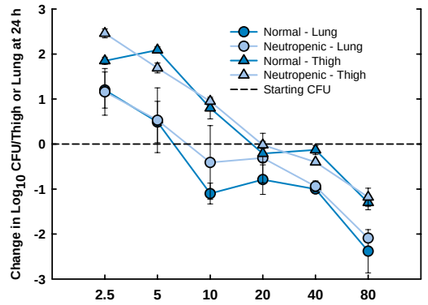
Figure 2. Effect of single doses of BC-3781 on time course of antimicrobial activity with *S. aureus* ATCC 25923 in the thighs of neutropenic mice



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 neutropenic mice. In these experiments, the lungs and the thighs were infected in the same mice. The dose-response relationships for BC-3781 against this organism in the thighs and lungs of neutropenic and normal mice are shown in Figure 3. The presence of white blood cells (WBC) enhanced slightly the activity of BC-3781 against *S. pneumoniae* lung infection, but no impact of WBC on efficacy could be observed against thigh infection.

Figure 3. 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



However the drug appeared to have enhanced potency (3-fold) in the lung compared to the thigh in both neutropenic and normal mice, respectively.

Indices 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. Table 1 lists the coefficient of determination observed for the relationship between efficacy and each PK/PD inices. The best correlations were observed with the 24 h AUC/MIC followed by the $T_{>MIC}$ ratios.

Table 1. Coefficients of determination for relationship between efficacy and PK/PD indices for BC-3781 against two organisms in the thighs of neutropenic mice

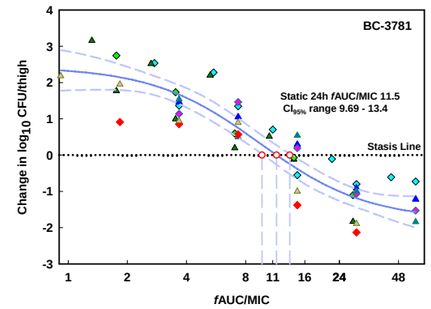
Organism	Dosing Interval	Coefficients of Determination		
		C_{max}/MIC	24h AUC/MIC	$T_{>MIC}$
S. pneumoniae	**3, 6, 12, 24 h**	**57,9 %**	**79.9 %**	**68.1 %**
S. aureus	**3, 6, 12, 24 h**	**59,4 %**	**78.3 %**	**77.5 %**

In vivo Breakpoints Associated with Efficacy

The *in vivo* pharmacodynamic activity of BC-3781 was evaluated against *S. aureus* as a predominant SSSI pathogens. The activity of q12 dosing regimens of BC-3781 was investigated in two PK/PD murine thigh infection studies with similar set up. The group of *S. aureus* include two MSSA and six MRSA strains, including hospital acquired 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, the sigmoid curve fit of mean and $CI_{95\%}$ are depicted in Figure 4. The 24 h *f*AUC/MIC necessary to produce a net static effect against *S. aureus* was 11.5 with a 95 % confidential interval band of 9.69 to 13.4 .

Figure 4. Inhibitory E_{max} model and 95 % confidence interval of the combined dataset of 24 h *f*AUC/MIC as a function of change in CFU/thigh for *S. aureus* isolates



Conclusions

The above studies have characterized the *in vivo* pharmacodynamic activity of BC-3781 against various strains of *S. pneumoniae* and *S. aureus*:

- The drug appears to exhibit time-dependent killing but also produces modest *in vivo* PAEs.
- The 24 h AUC/MIC and the $T_{>MIC}$ were the PK/PD indices most important for efficacy.
- The magnitude of the 24 h *f*AUC/MIC required for the various strains of *S aureus* was 11.5 ($CI_{95\%}$ = 9.69-13.4).
- The drug 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 slight effect in enhancing the activity of the drug.