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In Vivo Pharmacodynamic Activity of BC-3205, a Novel Pleuromutilin Derivative

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Abstract

Background: BC-3205 is a novel semi-synthetic pleuromutilin derivative that inhibits prokaryotic protein synthesis. BC-3205 is in early stage of clinical development for oral treatment of skin and skin structure infections (SSSI) and community-acquired pneumonia (CAP). We used the neutropenic murine thigh infection model to characterize the *in vivo* pharmacodynamic activity of BC-3205 against *S. aureus* (SA) and *S. pneumoniae* (SP).

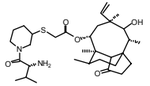
Methods: The number of organisms at the start of therapy in the following studies varied from $10^{6.4}$ to $10^{7.7}$ cfu/thigh or lung of either *S. aureus* or *S. pneumoniae*. Treatment comprised either single doses (10-160 mg/kg) or was fractionated into 2, 4 or 8 daily doses. The impact of infection site was examined using the lung and thigh model. The E_{max} dose-response model was used to determine the daily dose required to produce a net bacteriostatic effect. Plasma protein binding was determined *in vitro* using equilibrium dialysis.

Results: The effect of single doses of 10, 40, and 160 mg/kg of BC-3205 (administered s.c.) on *in vivo* killing and re-growth of SA or SP were examined. BC-3205 appears to exhibit time-dependent killing with prolonged postantibiotic effects (PAE), about 6 hours for SP, and over 8.5 hours for SA. The 24 h AUC/MIC and Cmax/MIC were the PK/PD indices most important for efficacy. There was no difference in activity of the drug in immuno-competent mice in comparison to immuno-compromised mice. The drug appeared to have 2-fold higher potency in the lung than the thigh. The *in vitro* plasma protein binding in mice was 94%.

Conclusions: The magnitude of the free 24 h AUC/MIC required for the various strains of *S. pneumoniae* and *S. aureus* ranged from 4.05 to 20.7. Accordingly, a daily dose achieving a 24 h *f*AUC/MIC value of about 13 is predicted to be efficacious in 90% of the patients.

Introduction

Chemical Structure of BC-3205



Following studies were performed to characterize the *in vivo* pharmacodynamic properties of BC-3205, a novel pleuromutilin antibiotic. The influence of dosing regimen on the *in vivo* efficacy of BC-3205 was determined in an 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-3205: 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 PK of BC-3205 in thigh infected neutropenic Swiss ICR mice were determined by HPLC/MS/MS on serum samples collected over six hours.

***In Vivo* Efficacy:** The neutropenic murine thigh infection model and the lung infection model was used to address the *in vivo* efficacy of subcutaneously dosed BC-3205. 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 nine *S. pneumoniae* and seven *S. aureus* strains inducing experimental infection was in the range of $10^{6.4}$ to $10^{7.7}$ CFU/thigh or lung.

Results

Table 1: Pharmacokinetic parameters of BC-3205 after subcutaneous administration

Dose [mg/kg]	t_{max} [h]	C_{max} [µg/ml]	$AUC_{0\text{-}6\ h}$ [µg.h/l]	$AUC_{0\text{-}24\ h}$ [µg.h/l]	Half-life [h]
10	0.5	1.340	1.94	1.95	0.62
40	1.0	3.208	13.7	20.1	3.17

The plasma protein binding determined by equilibrium dialysis at a concentration of 3 µg/ml was reported to be 94 % in the mouse.

The effect of single doses of 10, 40 and 160 mg/kg of BC-3205 on the *in vivo* killing and regrowth of *S. pneumoniae* ATCC 10813 is shown in Figure 1. Each point represents the mean of 2-3 mice. The rate of killing of the strain of *S. pneumoniae* was not increased by higher drug concentrations. However, regrowth of *S. pneumoniae* began at 2 h at 10 mg/kg and at 12 h with 40 mg/kg; regrowth did not occur at the highest dose. Free drug plasma concentrations were estimated to fall below the MIC at about 5.8 h with the 40 mg/kg dose. This suggests that the *in vivo* post antibiotic effect (PAE) is around 6.2 h.

The effect of single doses of 10, 40 and 160 mg/kg of BC-3205 on the *in vivo* killing and regrowth of *S. aureus* ATCC 29213 is shown in Figure 2. Killing was minimal with *S. aureus* but only the 40 and 160 mg/kg doses produced free plasma concentrations above the MIC (about 1.4 and 15.5 h, respectively). With the 40 mg/kg dose regrowth of the organism began after 6 h resulting in an *in vivo* PAE of 4.5 h. No regrowth occurred with the 160 mg/kg dose over the 24 h study period. This would result in an *in vivo* PAE of over 8.5 h.

Figure 1: Effect of single dose of BC-3205 on time course of antimicrobial activity with *Streptococcus pneumoniae* ATCC 10813 in the thighs of neutropenic mice

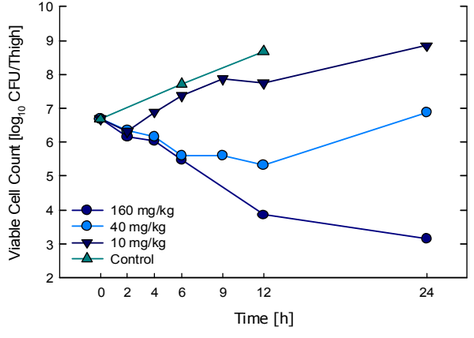
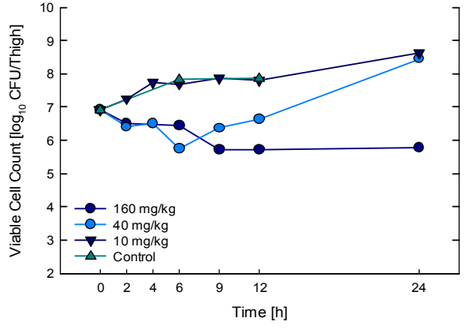


Figure 2: Effect of single dose of BC-3205 on time course of antimicrobial activity with *Staphylococcus aureus* ATCC 29213 in the thighs of neutropenic mice



Parameters Correlating with Efficacy

To determine which PK/PD index correlated best with efficacy of BC-3205 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.

Free drug concentrations were used to calculate the magnitude of the various PK/PD indices. Table 2 lists the coefficient of determination observed for the relationship between efficacy and each PK/PD parameter. The best correlations were observed with the 24 h AUC/MIC followed by the C_{max}/MIC ratios.

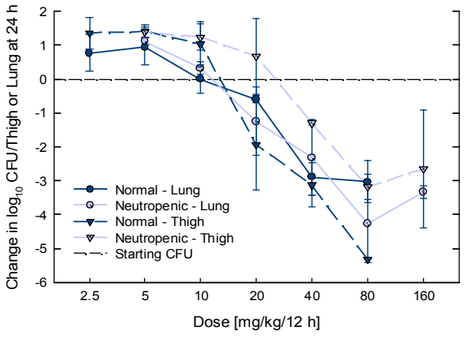
Table 2: Coefficients of determination for relationship between efficacy and PK/PD indices for BC-3205 against two organisms in the thighs of neutropenic mice

Organism	Data Used	Coefficients of Determination		
		C_{max}/MIC	24h AUC/MIC	$Time_{>MIC}$
S. pneumoniae	3, 6, 12, 24 h	74.5 %	74.7%	20.4 %
S. aureus	3, 6, 12, 24 h	82.0 %	77.1 %	26.9 %

Impact of Neutrophils and Site of Infection on Activity of BC-3205

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 relationships for BC-3205 against this organism in the thighs and lungs of neutropenic and normal mice are shown in Figure 3. The presence of white blood cells (WBCs) enhanced the activity of BC-3205 against *S. pneumoniae* only slightly. The drug appeared to have enhanced potency (about 2-fold) in the lung compared to the thigh in both normal and neutropenic mice.

Figure 3: Dose-response relationships for 12-hourly dosing of BC-3205 (s.c.) against *Streptococcus pneumoniae* ATCC 10813 in the thighs and lungs of normal and neutropenic mice



Parameters Correlating with Efficacy

To determine if the 24 h AUC/MIC required for a static effect was similar for multiple pathogens, the activity of 12-hourly dosing regimens of BC-3205 against nine strains of *S. pneumoniae* and seven strains of *S. aureus* was studied. As illustrated in Table 3 the extent of bacterial killing in neutropenic mice was excellent for *S. pneumoniae* and moderate for *S. aureus*. The total AUC/MIC values 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 6% unbound BC-3205 was used for calculations of 24 h *f*AUC/MIC. The 24 h *f*AUC/MIC values at static dose varied from 4.23 to 13.7 for *S. pneumoniae* with a mean value of 7.00 and from 4.05 to 20.70 for *S. aureus* with a mean value of 12.80. One of the pneumococcal strains was intermediate to penicillin and seven strains were resistant to penicillin. The group of *S. aureus* includes four MSSA and three MRSA strains, two being hospital acquired and one community acquired.

Table 3: The static dose, maximum killing and free 24 h AUC/MIC ratio required for a static effect of BC-3205 (bid) against 16 organisms

Organism	MIC [µg/ml]	Comments	Static Dose [mg/kg/12 h]	24 h *f*AUC/MIC Static Dose	Maximal Killing log_{10}CFU/ Thigh
S. pneumoniae ATCC 10813	0.06	PSSP	21.4	13.7	-2.24
S. pneumoniae ATCC 145	0.06	PRSP	11.1	4.64	-3.45
S. pneumoniae ATCC 146	0.03	PRSP	9.36	7.26	-3.49
S. pneumoniae CDC 1020	0.06	PRSP	12.2	5.42	-3.15
S. pneumoniae CDC 1199	0.03	PRSP	10.7	8.18	-3.85
S. pneumoniae CDC 1293	0.06	PRSP	16.5	8.90	-3.65
S. pneumoniae CDC 1325	0.06	PRSP	10.5	4.23	-3.46
S. pneumoniae CDC 1329	0.03	PRSP	7.62	5.97	-5.07
S. pneumoniae CDC 1396	0.06	PISP	11.3	4.77	-3.11
S. aureus ATCC 29213	0.12	MSSA	15.6	4.05	-0.68
S. aureus ATCC 25923	0.12	MSSA	23.4	7.91	-2.05
S. aureus ATCC 6538P	0.12	MSSA	41.9	20.7	-2.20
S. aureus ATCC Smith	0.12	MSSA	33.4	14.2	-2.58
S. aureus Wis-1	0.12	HA-MRSA	32.1	13.4	-2.97
S. aureus UW 307109	0.12	CA-MRSA	38.3	17.9	-2.69
S. aureus R2527	0.06	HA-MRSA	19.3	11.5	-2.54

Conclusions

The above studies have characterized the *in vivo* pharmacodynamic activity of BC-3205 against various strains of *S. pneumoniae* and *S. aureus*:

- The drug appears to exhibit time-dependent killing but also produces prolonged *in vivo* PAEs.
- The 24-hr AUC/MIC and the Cmax/MIC were the PK/PD indices most important for efficacy. The magnitude of the 24 h AUC/MIC of free drug required for the various strains of *S. pneumoniae* and *S. aureus* ranged from 2.70 to 11.9.
- The presence of WBCs had only a slight effect in enhancing the activity of the drug in both the thighs and the lungs. The drug was more potent (about 2-fold) in the lung compared to the thigh in both neutropenic and normal mice.