Poster No. A-013

Comparative Pharmacodynamics of BC-3781 in Murine *Streptococcus Pneumoniae* Thigh and Lung Infection Models

W.W. Wicha, E. Fischer, B. C. Kappes, Z. Ivezic-Schoenfeld
Nabriva Therapeutics AG, Vienna, Austria

Nabriva Therapeutics AG
Leberstrasse 20
A-1110 Vienna
Austria
www.nabriva.com
+43-1-74093-0
wolfgang.wicha@nabriva.com

ABSTRACT

Background: BC-3781 is a pleuromutilin antibiotic that inhibits prokaryotic protein synthesis. It is currently undergoing clinical development for intravenous and oral treatment of acute bacterial skin and skin structure infections (ABSSSI) and is under consideration for study in community-acquired bacterial pneumonia (CABP). BC-3781 has completed a phase 2 study in patients with severe ABSSSI caused by Gram-positive pathogens. Here, we describe investigations into the pharmacodynamics of BC-3781 against clinical isolates of *S. pneumoniae* in neutropenic murine thigh and lung infection models.

Methods: Thighs or lungs of neutropenic mice infected with *S. pneumoniae* (n = 6; MIC 0.12-0.5 µg/ml) were treated twice daily with escalating BC-3781 doses from 2.5 to 320 mg/kg/day. At the start of therapy (EC), mice had between 5.5 to 6.3 $\log_{10}$ CFU/tissue. The 24 h *f*AUC/MIC ratio achieving a net static effect and other curve parameter were extrapolated following an inhibitory sigmoid E_{max} curve-fit to response data.

Results: The pharmacodynamics of BC-3781 were similar for all *S. pneumoniae* strains showing a good *in vitro/in vivo* correlation regardless of site of infection. The mean 24 h *f*AUC/MIC ratio derived from the thigh infection model against 6 *S. pneumoniae* clinical isolates was 6.40 (CI95% 5.48-7.50). This breakpoint determined was 2-fold lower than the target obtained previously against a set of *S. aureus* strains using the same experimental setup. Since *S. pneumoniae* is the most frequent causative organism in community acquired respiratory bacterial infections, the same pathogens used in the thigh infection model were employed in a murine lung model to draw a comparison on the PD of BC-3781 at different sites of infection. The mean 24 h *f*AUC/MIC ratio for stasis in the lung infection model was 0.76 or 8.4-fold below the comparable endpoint in the thigh infection. By comparing both sigmoid E_{max} curves a 4-fold enhanced therapeutic activity of BC-3781 in lung tissues was observed.

Conclusions: BC-3781 showed good activity against *S. pneumoniae* infections with an enhanced activity in lung tissues, compared to thigh. The PKPD information obtained in this study would support a study against respiratory tract infections and provides a robust basis for target attainment analysis.

INTRODUCTION

A murine thigh infection model as described by W.A. Craig was employed to investigate the efficacy of BC-3781 against *S. pneumoniae* isolates covering relevant BC-3781 MIC range from 0.12 to 0.5 µg/mL. MIC_{90} for BC-3781 is 0.25 µg/mL, irrespective of penicillin-susceptibility (n = 1473; SENTRY 2010).[1] Based on the results of the published study by W.A. Craig *et.al*,[2] the study described herein was initiated to better support the target attainment required for the dose rationale focusing on community-acquired bacterial pneumonia (CABP). Following these PK/PD studies using the thigh infection model, selected bacterial strains with confirmed virulence were used in a murine lung infection model to investigate the pharmacodynamics of BC-3781 among pathogens and infection sites.

METHODS

Animals: Female mice weighing 20-30 grams were allowed to acclimate approximately one week and were utilized throughout the experiments. Prior to the infection, mice were rendered neutropenic.

Bacterial and minimal inhibitory concentrations (MIC): MIC were determined by CLSI broth microdilution method.[3] From the list below, the strains used for PK/PD studies (n = 6) were selected to cover the relevant MIC range for BC-3781 against *S. pneumoniae* and depending on their virulence and therefore suitability in this model.

Strain ID	Category	MIC [µg/mL]
B1378	penicillin-intermediate	0.12
B1379	penicillin-intermediate	0.12
B1382	penicillin-susceptible	0.25
B1383	penicillin-resistant, macrolide-resistant	0.25
B1385	penicillin-susceptible	0.5
B1386	penicillin-intermediate, macrolide-resistant	0.5

Neutropenic mouse thigh infection model: Bacterial suspensions were prepared from overnight culture of *S. pneumoniae* incubated on blood agar plates at 37 °C. Mice were challenged with 7.05-7.48 $\log_{10}$ CFU/mL. The size of the inoculum of each strain was adjusted to achieve 5.5-6.2 $\log_{10}$ CFU/thigh at start of therapy. 2 h p.i. mice were given doses between 10 and 320 mg/kg/day BC-3781 s.c. twice daily. 24 h after the first treatment the animals were sacrificed and CFU/thigh were evaluated.

Neutropenic mouse lung infection model: Each animal was infected by placing 50 µL of 6.80-7.64 $\log_{10}$ CFU/mL inoculum onto the tip of the nares. Animals were allowed to inhale the bacterial suspension as small droplets and then placed back into their cages for recovery and observation prior to dosing. The treatment started 2 h p.i. To evaluate the dose response relationship of BC-3781 doses of 2.5 to 320 mg/kg s.c. were given twice daily. At the end of the treatment period (24 h after start of therapy) in all experiments the lungs were removed aseptically the number of viable organisms at the infection site was determined by agar plating.

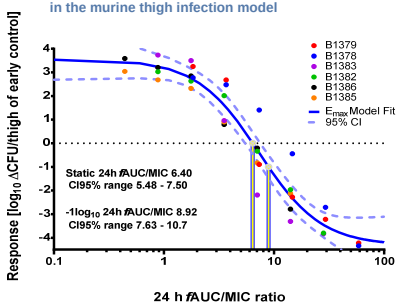
Data Analyses: The response (ΔCFU/tissue) following 24 h treatment at exposure levels of various doses was compared to the bacterial burden before onset of treatment (early control; EC) and the ΔCFU of the untreated control group (late control; LC) using an Mann-Whitney rank sum test. The escalated doses were translated into unbound drug exposure values based on values reported in an earlier study and normalized by individual strain MIC values.[2]

The 24 h *f*AUC/MIC ratio achieving a net static effect (bacteriostatic effect), 24 h *f*AUC/MIC ratio reducing bacterial burden by 1 $\log_{10}$ (1 $\log_{10}$ kill CFU/tissue), the effective 24 h *f*AUC/MIC ratio reducing 50 % of the bacterial burden (EC_{50}), the effective 24 h *f*AUC/MIC ratio reducing 20 % of the bacterial burden (EC_{20}) and the effective 24 h *f*AUC/MIC ratio reducing 80 % of the bacterial burden (EC_{80}) were determined using an inhibitory sigmoid E_{max} model (logistic function, Origin Pro 8G).

RESULTS

At the start of therapy (EC, early control), mice had between 5.51 to 6.32 $\log_{10}$ CFU/thigh. In untreated animals (LC, late control) pathogens reached bacterial burdens of at least 9 $\log_{10}$ CFU/thigh. In three tested isolates (B1382, B1383, B1386) untreated control animals showed mortality within the 24 h exceeding bacterial burden of 9.6 $\log_{10}$ CFU/thigh. The mean 24 h *f*AUC/MIC ratio (CI95%) for stasis determined using a nonlinear curve fit function over all six strains was 6.40 (5.48-7.5) showing a good *in vitro/in vivo* correlation (MIC–static dose relationship) as depicted in Figure 1.

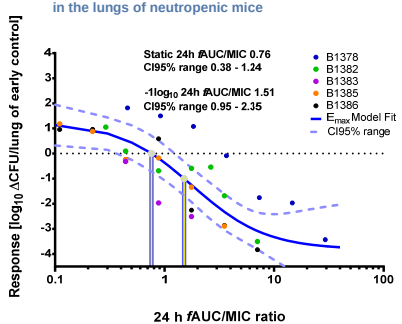
Figure 1. PK/PD relationship of BC-3781 against *S. pneumoniae* in the murine thigh infection model



Five of the strains used in the thigh infection model were used to induce a bronchopneumonia in mice. Except one strain (B1379; excluded from the curve fit analysis) all isolates resulted in a stable lung infection in neutropenic mice. At start of therapy the bacterial burden was between 5.64 and 6.05 $\log_{10}$ CFU/lung. The untreated groups of the five remaining strains reached $\log_{10}$ CFU/lung values of 6.20 to 7.98 within 24 h.

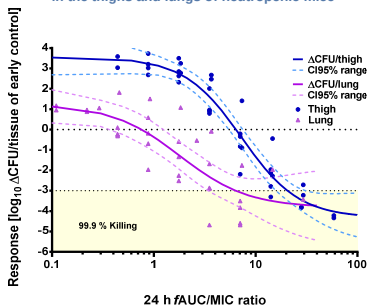
The mean dose-response curve, the corresponding 95 % confidence interval (CI95%), and stasis and 1 $\log_{10}$ kill 24 h *f*AUC/MIC values following treatment with escalating doses of BC-3781 against the five *S. pneumoniae* isolates are depicted in Figure 2.

Figure 2. PK/PD relationship of BC-3781 against *S. pneumoniae* in the lungs of neutropenic mice



To investigate the efficacy of BC-3781 on site of infection (thigh vs. lung) the endpoints (stasis, 1 $\log_{10}$ kill ED_{20}, ED_{50} and ED_{80}) were used to compare the two sigmoid response curves (Figure 3).

Figure 3. Comparative E_{max} model fits and CI95% of 24 h *f*AUC/MIC as a function of change in CFU against multiple strains of *S. pneumoniae* in the thighs and lungs of neutropenic mice



In all determined endpoints BC-3781 showed an increased efficacy of at least 3.4-fold against lung infections compared to response in the thigh infections (Table 1). Considering the most robust endpoint, the EC_{50}, BC−3781 showed a 4-fold higher efficacy against lung infections compared to thigh infection caused by *S. pneumoniae*.

Table 1. Impact of site of infection on the activity of BC-3781

Endpoint	Thigh	Lung	Enhancement Ratio
Stasis	6.40	0.76	8.4-fold
-1$\log_{10}$ kill	8.92	1.51	5.9-fold
EC_{20}	2.96	0.61	4.9-fold
EC_{50}	7.28	1.80	4.0-fold
EC_{80}	17.9	5.25	3.4-fold

CONCLUSIONS

- BC-3781 showed good activity against *S. pneumoniae* infections with an enhanced activity in lung tissues, compared to thigh
- This study indicates that BC-3781 warrants further investigation against bacterial respiratory tract infections
- The PK/PD information obtained in this study provides a robust basis for target attainment analysis

SELECTED REFERENCES

1. Paukner, S., Sader, H. S., Ivezic-Schoenfeld, Z., Jones, R. N., Antimicrobial activity of the pleuromutilin antibiotic BC-3781 against bacterial pathogens isolated in the SENTRY Antimicrobial Surveillance Program (2010) *Antimicrob. Agents Chemother.* **57**(9) (2013) 4489-4495
2. Craig W.A., D. Andes, Z. Ivezic Schoenfeld, R. Novak, W.W. Wicha. 2010. *In vivo* pharmacodynamic activity of BC-3781. 50th ICAAC, Boston, USA, abstract F1-2108
3. Clinical and Laboratory Standards Institute (2009). *M07-A8. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard: eighth edition*, Wayne, PA: CLSI

53rd Interscience Conference on Antimicrobial Agents and Chemotherapy, Denver, CO, September 10-13, 2013