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In Vivo Antimicrobial Activity of MGB-BP-3, a New Class of Antibacterial Agent, in Murine Infection Models

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ABSTRACT

Background: MGB-BP-3 is a new class of antibiotic that binds selectively to the Minor Groove of DNA and has shown strong *in vitro* bactericidal activity against aerobic Gram-positive bacteria, including susceptible and resistant *Streptococci*, *Staphylococci*, and *Enterococci* strains. This study assesses the *in vivo* pharmacokinetics (PK) and pharmacodynamics (PD) of MGB-BP-3.

Methods: The murine thigh infection model was used to investigate the ED50 and the PD of MGB-BP-3. Naïve mice were rendered neutropenic, inoculated intramuscularly with 106 - 107 CFU of *S. pyogenes, S. pneumoniae* or MRSA, and treated IV with 20-100 mg/kg MGB-BP-3, after which, concentration-time curves were generated. 6 h post treatment, bacterial load (CFU) in the thigh was determined by spot tissue homogenates. The PD of MGB-BP-3 was investigated following single or multiple dosing, ranging from 20 - 100 mg/kg. The tissue spots were incubated on agar plates, and colony counts were determined at 24 h. *In vivo* efficacy of optimal dosing was verified in the murine pneumonia model with *S. pneumoniae*.

Results: The ED50 of MGB-BP-3 was determined to 62 mg/kg, 50 mg/kg and 51 mg/kg respectively for *S. pyogenes*, *S. pneumoniae* and MRSA in the thigh infection model. Fractionated dosing studies revealed that efficacy against *S. pneumoniae* and MRSA correlated equally well to T>MIC and AUC24/MIC at an assumed protein binding of 80%. 90% of max efficacy against MRSA was estimated to be reached at AUC24/MIC = 288 h, and against *S. pneumoniae* at AUC24/MIC = 263 h. The most potent effect of MGB-BP-3 against *S. pneumoniae* was observed when dosing mice 4 x 60 mg/kg, resulting in a 5.1 log10 reduction of bacterial load compared to vehicle treatment, whereas 4 x 20 mg/kg resulted in a 2.1 log10 reduction. Similar dosing scenarios, 4 x 20 mg/kg, 4 x 40 mg/kg and 3 x 60 mg/kg, resulted in a potent effect in the pneumonia model with a 2.1, 2.3 and 2.7 log10 reduction of bacterial load respectively, compared to the vehicle.

Conclusion: MGB-BP-3 showed a potent antibacterial *in vivo* effect against *S .pneumoniae* and MRSA in experimental infection models. Efficacy correlated to T>MIC and AUC24/MIC.

INTRODUCTION

MGB-BP-3 is a new class of antibiotic, belonging to the DNA Minor Groove Binder group, that has very strong antibacterial activity against all susceptible and multi-resistant Gram-positive pathogens tested, including methicillin-resistant and susceptible *Staphylococcus* species, pathogenic *Streptococcus* species, Vancomycin-Resistant and susceptible *Enterococcus* and *Clostridium difficile.*

MGB-BP-3 was originally developed by scientists at the University of Strathclyde [1]. It has the architecture of a typical minor groove binder and is based upon the structure of naturally occurring product, Distamycin (Figure 1).

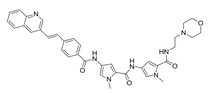


Figure 1. Molecular structure of MGB-BP-3

Its oral formulation is currently in a clinical Phase I study for the treatment of *C. difficile* infections, and its intravenous formulation is in the final stages of preclinical development for the treatment of hospital acquired Gram-positive infections.

In this group of experiments, we investigated *in vivo* activity of MGB-BP-3 against *Streptococcal* and *Staphylococcal* bacteria in various mouse infection models. The aim was to quantify MGB-BP-3's antibacterial activity in live organisms and assess the correlation between activity and plasma concentrations.

METHODS

Assessment of pharmacokinetic profile of MGB-BP-3: Mice were injected IV with a single dose of MGB-BP-3 in the range 20 - 100 mg/kg. Blood samples were collected from 15 minutes to 24 h after injection. Plasma concentrations were quantified using an optimised and validated HPLC method.

Assessment of Minimum Inhibitory Concentration (MIC) for *S. pneumoniae* and *S. aureus*: The MIC of MGB-BP-3 against *S. aureus* (MRSA) and *S. pneumoniae* was determined by using a broth micro dilution method in accordance with CLSI guidelines. The tested range was 0.031 - 32 µg/ml.

Quantification of the antibacterial effect of MGB-BP-3 in mouse thigh model: Assessment of efficacy of MGB-BP-3 against *S. pneumoniae* and *S. aureus* (MRSA) in the murine neutropenic thigh infection model was investigated following IV administration. Mice were injected intraperitoneally with cyclophosphamide at 200 mg/kg (Day -3) and 100 mg/kg (Day -1). Mice were then inoculated intramuscularly with approximately 1-5 x10^7 CFU/ml of *S. pneumoniae* or MRSA suspension in the left thigh. Mice were treated with a single or fractionated IV dose of MGB-BP-3 at 10-100 mg/kg or vehicle over approximately 1 min, 1h post infection. The positive control for *S. pneumoniae* was subcutaneous penicillin, and IV vancomycin for MRSA and *S. pyogenes.* The colony counts in thighs were determined at 1h for vehicle, 6h for *S pyogenes*, and 24h for *S pneumoniae* post infection for MGB-BP-3.

PK/PD modelling to identify the optimal dosing regimen: The purpose of this study was to select, by means of mathematical modelling, a set of suitable MGB-BP-3 dosing regimens to obtain optimal separation of the PK/PD indices for future pharmacodynamic studies with MRSA and *S. pneumoniae*.

Pharmacokinetic indices (Cmax, AUC_{24} and terminal elimination half-life T1/2) were determined by non-compartmental modelling with the initESTIM program in the PKPDsim software package [2]. A linear two-compartment model (Figure 2) was fitted to concentration-time data. The pharmacokinetic indices were then calculated by simulation of a selected dosage regimen with the fitted compartment model. The NPAG population parameter estimation program [3, 4] was used to fit the compartment model.

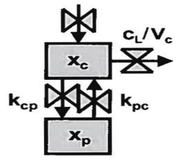
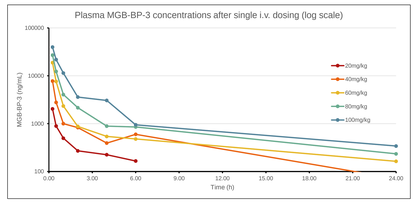


Figure 2. Linear compartment model with four PK parameters: Vc, CL, kcp, kpc· Xs denote the amount of drug in the various compartments. The modelled concentration of drug in plasma is given as conc = Xc/Vc.

Efficacy of MGB-BP-3 in murine pneumonia disease model. Mice were inoculated intra-nasally with *Streptococcus pneumoniae* D39 and treated multiple times IV with MGB-BP-3 over 24 h. The colony counts in lungs were determined at the start of treatment and 24 h after the start of treatment.

RESULTS

The pharmacokinetics of MGB-BP-3 in mice showed a rapid initial redistribution followed by slow elimination. There was substantial 'between subject' variability.



Dose (mg/kg)	C_{max} (mg/mL)	AUC24 (h.mg/L)	T1/2 (h)
20.00	2.00	3.80	5.70
40.00	7.80	14.00	7.90
60.00	19.00	22.00	12.00
80.00	27.00	35.00	10.00
100.00	40.00	57.00	7.80

MGB-BP-3 showed dose dependent activity against *S. pyogenes, S. pneumoniae* and *S. aureus (*MRSA) in mouse thigh model (Figures 3-5).

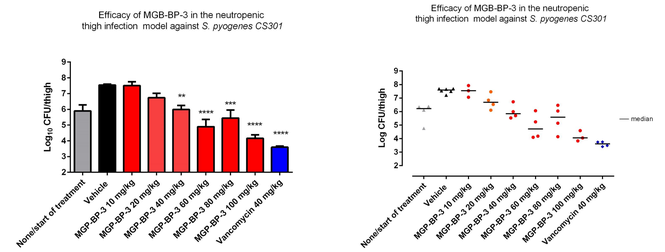


Figure 3 – Efficacy of MGB-BP-3 in the thigh infection model against *S. pyogenes.*

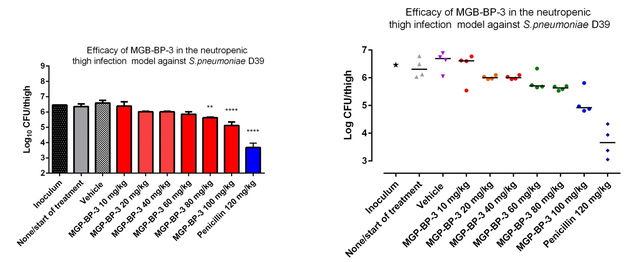


Figure 4 – Efficacy of MGB-BP-3 in the thigh infection model against *S. pneumoniae*

RESULTS

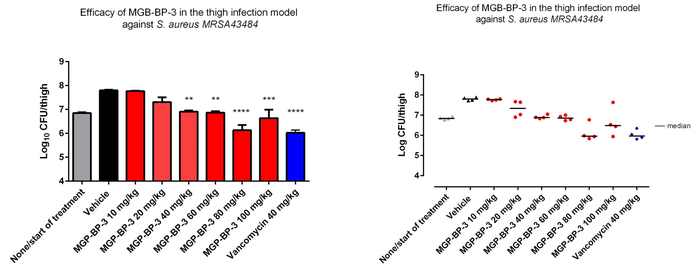


Figure 5 – Efficacy of MGB-BP-3 in the thigh infection model against *S. aureus (MRSA).*

PK/PD modelling showed high correlation of %T>MIC and AUC24/MIC indices with efficacy for selected dosage regimens obtained by non-compartmental modelling of the free drug concentration in serum. Plasma protein binding was unknown and taken to be 0% in this calculation. MIC_{Spneu}= 0.125 mg/L MIC_{MRSA} = 0.25 mg/L

Total dose (mg/kg)	T>MICa (%)	AUC24/MICa (h)	Cmax/MICa (-)	T>MICb (%)	AUC24/MICb (h)	Cmax/MICb (-)
20 x 1	35	31	25	12	15	12
40 x 1	95	113	113	76	57	56
60 x 1	100	176	275	79	88	138
80 x 1	100	284	374	98	142	187
100 x 1	100	457	458	100	229	229
20 x 2	71	53	25	24	26	12
80 x 2	100	483	374	100	241	187
20 x 4	100	83	25	48	41	12
40 x 4	100	256	113	100	128	56
60 x 4	100	517	275	100	259	138

Murine pneumonia disease model confirmed dependency of MGB-BP-3 efficacy against *S. pneumoniae* on AUC24/MIC assessed by PK/PD model (Figure 6).

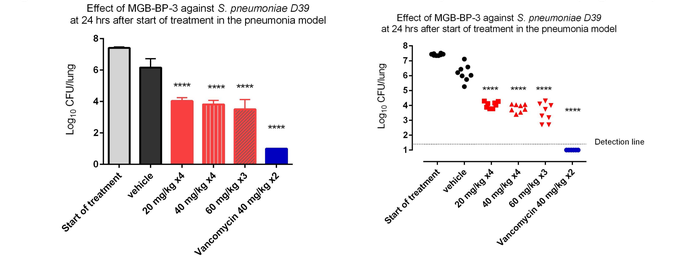


Figure 6 – Efficacy of MGB-BP-3 against *S. pneumoniae* in the murine pneumonia disease model.

CONCLUSION

- MGB-BP-3 pharmacokinetic profile showed a rapid initial redistribution followed by slow elimination. The half-life was in the range of 6 to 12 hours.
- MGB-BP-3 showed high activity against *Streptococcus pyogenes, Streptococcus pneumonia* strains, and *Staphylococcus aureus* (MRSA) in the mouse thigh model.
- PK/PD modelling showed that %T>MIC and AUC24/MIC are highly correlated with efficacy of MGB-BP-3.
- The accurate assessment of plasma protein binding will allow inclusion of this parameter into the calculation of the PK/PD relationship and selection of a suitable MGB-BP-3 dosing regimens for future pharmacodynamic studies.
- MGB-BP-3 showed high activity in the mouse pneumonia disease model.

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