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Evaluation of *in vitro* antibacterial activity of MGB-BP-3, a new class of antibacterial

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ABSTRACT

Objectives: MGB-BP-3 (MGB) is a new class of antibacterial agent discovered at the University of Strathclyde. It binds selectively to the minor grooves of DNA. We investigated the spectrum of antibacterial activity against a panel of bacteria. The antibacterial profile of MGB was investigated further in susceptible bacteria to determine MIC_{50}/MBC_{50} and MIC_{90}/MBC_{90}, killing activity, and the potential for development of resistance.

Methods: The CLSI methodology was used throughout the experiments. MIC was assessed in a panel of 52 Gram-positive and 34 Gram-negative bacteria and compared to standard antibiotics. The MIC50/MBC50 and MIC90/MBC90 were determined in a panel of 210 Gram-positive isolates consisting of vancomycin susceptible and resistant enterococci, meticillin susceptible and resistant Staphylococcus aureus and S. epidermidis, and a group of streptococci. The bactericidal activity of MGB was determined measuring in vitro killing activity (time kill and bactericidal index) against vancomycin-susceptible E. faecalis, meticillin-susceptible S. aureus and Streptococcus pyogenes. These strains were also used to assess the propensity to develop resistance to MGB and a comparator (fusidic acid) by mutation frequency following serial passage over 14 days at sub-inhibitory antimicrobial concentrations (0.5 x MIC).

Results: MGB showed high activity against all Gram-positive isolates tested, including vancomycin and meticillin resistant strains. At 0.5mg/L, MGB inhibited 86.5% of all tested Gram-positive isolates, compared to 43.4% tested with vancomycin. With Gram-negative isolates the only MGB activity observed was against N. meningitidis (MIC 0.25 mg/L) and two M. catarrhalis strains (MIC of 0.5 mg/L and 2 mg/L).The data indicate that MGB is bactericidal against staphylococci and streptococci, whilst it is bacteriostatic to enterococci. Resistance tests showed that E. faecalis can develop resistance against MGB, but there was no similar evidence for S. aureus and S. pyogenes.

Conclusion: MGB has been shown to have very strong inhibitory activity against all tested Gram-positive bacteria including vancomycin and meticillin resistant strains. Its activity against staphylococci and streptococci is bactericidal, and bacteriostatic against enterococci. The possibility of developing resistance to MGB was confirmed with E. faecalis but not with S. aureus and S. pyogenes

INTRODUCTION

MGB is a new class antibacterial agent, under preclinical development at MGB Biopharma; it binds selectively to the Minor Groove of DNA.

We investigated the spectrum of antibacterial activity against a panel of Gram-positive and Gram-negative bacteria and fungi. The antibacterial profile of MGB was investigated further in susceptible bacteria to determine MIC_{50}/MBC_{50} and MIC_{90}/MBC_{90}, killing activity, and the potential for development of resistance.

METHODS

MIC testing

MIC was determined in all tests by broth microdilution for aerobic organisms and by agar dilution methodology for anaerobic organisms, according to CLSI M11-A7[1].

Initially MIC of MGB was assessed in a panel of 52 Gram-positive and 34 Gram-negative bacteria and 20 fungi and compared to standard antibiotics. Further MIC was assessed in 41 isolates from the Gram-positive bacteria, and 20 isolates *of C. difficile.*

MIC_{50}/MBC_{50} and MIC_{90}/MBC_{90} determinations

MIC_{50} and MIC_{90} together with MBC_{50} and MBC_{90} were assessed in a panel of 210 recent clinical isolates.

Analysis of the bactericidal activity

The killing activity of MGB and vancomycin were determined at 2, 4 and 8 fold MIC in vancomycin-susceptible *Enterococcus faecalis* , Meticillin-susceptible *Staphylococcus aureus and Streptococcus pyogenes* (Group A).

Mutation Frequency Resistance Testing

The frequency of occurrence of bacterial colonies showing resistance was determined, as evidenced by their ability to grow on Mueller Hinton agar (MHA) plates containing various multiples of the MIC for MGB and fusidic acid against *Enterococcus faecalis*, *Staphylococcus aureus* and *Streptococcus pyogenes*.

METHODS

Serial Passage Resistance Testing

Serial passage resistance development was performed assessing MIC data for MGB and fusidic acid against *Enterococcus faecalis*, *Staphylococcus aureus* and *Streptococcus pyogenes* over a 14 day period.

RESULTS

MIC Assessment showed that MGB possesses high antibacterial activity against all the Gram-positive bacteria tested (Table 1). At 0.5mg/L, MGB inhibited 86.5% of all tested Gram-positive isolates, compared to 43.4% tested with vancomycin. No activity was observed against fungi or Gram-negative bacteria, with the exception of *Neisseria meningitidis* and *Moraxella catarrhalis*.

Table 1. MIC values for MGB and vancomycin in a range of Gram-positive isolates.

	Gram-positive isolates	MGB-BP-3 MIC (mg/L)	Vancomycin MIC (mg/L)
1	EMRSA1 *Staphylococcus aureus* (MRSA) - SSCmec type 3	0.25	1
2	EMRSA15 *Staphylococcus aureus* (MRSA) - SSCmec type 4	0.25	0.5
3	EMRSA16 *Staphylococcus aureus* (MRSA) - SSCmec type 2	0.5	1
4	EMRSA3 *Staphylococcus aureus* (MRSA) - SSCmec type 1	0.25	1
5	*Staphylococcus aureus* - methicillin-resistant clinical isolate.	0.25	1
6	*Staphylococcus aureus* - multi-drug-resistant clinical isolate.	0.25	2
7	*Staphylococcus aureus* - teicoplanin-intermediate clinical isolate.	0.5	2
8	*Staphylococcus aureus* ATCC 25923 - antibiotic-susceptible type strain.	0.5	1
9	*Staphylococcus aureus* ATCC 29213 - antibiotic-susceptible type strain.	0.25	1
10	*Staphylococcus aureus* ATCC 43300 - methicillin-resistant type strain.	0.25	1
11	*Staphylococcus aureus HT2001254* (MRSA) - PVL positive	0.25	1
12	*Staphylococcus aureus* MU50 (MRSA) -VISA type strain	0.25	8
13	*Staphylococcus epidermidis* - antibiotic susceptible clinical isolate.	0.25	2
14	*Staphylococcus epidermidis* - methicillin-resistant clinical isolate.	0.5	2
15	*Staphylococcus haemolyticus* - antibiotic susceptible clinical isolate.	0.5	2
16	*Staphylococcus saprophyticus* - antibiotic susceptible clinical isolate.	0.25	1
17	*Enterococcus faecalis* - ATCC 29212 antibiotic-susceptible type strain.	1	4
18	*Enterococcus faecalis* - high-level gentamicin-resistant clinical isolate.	1	2
19	*Enterococcus faecalis* - vancomycin-resistant (VanA) clinical isolate.	2	>32
20	*Enterococcus faecalis* - vancomycin-resistant (VanB) clinical isolate.	0.5	>32
21	*Enterococcus faecalis* - vancomycin-susceptible clinical isolate.	0.25	4
22	*Enterococcus faecium* - vancomycin-resistant (VanA) clinical isolate.	0.5	>32
23	*Enterococcus faecium* - vancomycin-resistant (VanB) clinical isolate.	0.5	32
24	*Enterococcus faecium* - vancomycin-susceptible clinical isolate.	1	1
25	*Enterococcus gallinarum* - vancomycin-resistant (VanC) clinical isolate.	0.5	8
26	Group C *Streptococcus* - antibiotic-susceptible clinical isolate	0.25	0.5
27	Group C *Streptococcus* - macrolide-resistant clinical isolate	0.25	0.25
28	Group G *Streptococcus* - antibiotic-susceptible clinical isolate	0.25	0.12
29	Group G *Streptococcus* - macrolide-resistant clinical isolate	0.25	0.25
30	*Streptococcus agalactiae* - antibiotic-susceptable clinical isolate	0.25	0.5
31	*Streptococcus agalactiae* - macrolide-resistant clinical isolate	0.25	0.5
32	*Streptococcus bovis* - antibiotic-susceptible clinical isolate	0.25	0.5
33	*Streptococcus bovis* - macrolide-resistant clinical isolate	0.25	0.5
34	*Streptococcus constellatus* - antibiotic-susceptible clinical isolate	0.25	0.5
35	*Streptococcus constellatus* - macrolide-resistant clinical isolate	0.5	1
36	*Streptococcus mitis* - antibiotic-susceptible clinical isolate	0.25	0.5
37	*Streptococcus mitis* - macrolide-resistant clinical isolate	0.5	0.5
38	*Streptococcus oralis* - antibiotic-susceptible clinical isolate	0.25	0.5
39	*Streptococcus oralis* - macrolide-resistant clinical isolate	1	0.5
40	*Streptococcus pneumoniae* - ATCC 49619 antibiotic-susceptible strain.	0.25	0.25
41	*Streptococcus pneumoniae* - multi-drug resistant clinical isolate.	0.25	0.5
42	*Streptococcus pneumoniae* - penicillin-intermediate clinical isolate.	0.25	0.25
43	*Streptococcus pneumoniae* - penicillin-resistant clinical isolate.	0.25	0.12
44	*Streptococcus pneumoniae* - penicillin-susceptible clinical isolate.	0.25	0.12
45	*Streptococcus pyogenes* - antibiotic-susceptible clinical isolate.	0.25	0.5
56	*Streptococcus pyogenes* - Macrolide (MLS) resistant clinical isolate	0.25	0.5
57	*Streptococcus pyogenes* - Macrolide (M-type) resistance clinical isolate	0.25	0.5
48	*Streptococcus sanguis* - antibiotic-susceptible clinical isolate	0.5	0.5
49	*Streptococcus sanguis* - macrolide-resistant clinical isolate	0.5	0.5

MGB showed high activity against 20 isolates of *C. difficile* in comparison to clindamycin (Table 2).

Table 2. MIC determination for MGB and clindamycin against 20 *C. difficile* isolates.

C. Difficile Ribotype	MGB-BP-3 MIC mg/L (µM)	Clindamycin MIC mg/L (µM)
1	1 (1.6)	8 (17.3)
2	1 (1.6)	16 (34.6)
2	1 (1.6)	8 (17.3)
5	1 (1.6)	8 (17.3)
11	1 (1.6)	8 (17.3)
11	1 (1.6)	8 (17.3)
14	1 (1.6)	8 (17.3)
14	1 (1.6)	8 (17.3)
14	1 (1.6)	8 (17.3)
18	1 (1.6)	8 (17.3)
23	1 (1.6)	8 (17.3)
27	1 (1.6)	16 (34.6)
27	1 (1.6)	4 (8.7)
27	1 (1.6)	8 (17.3)
50	1 (1.6)	8 (17.3)
50	1 (1.6)	8 (17.3)
54	1 (1.6)	8 (17.3)
106	1 (1.6)	8 (17.3)
106	1 (1.6)	16 (34.6)
176	1 (1.6)	8 (17.3)
176	1 (3.2)	8 (17.3)
ATCC 700057	1 (1.6)	8 (17.3)

MIC_{50}/MBC_{50} and MIC_{90}/MBC_{90} determinations showed that MGB has pronounced bactericidal activity against *Staphylococcus aureus* and especially *Streptococcus pyogenes*. However, its activity against *Enterococcus faecalis* was found to be bacteriostatic (Table 3).

RESULTS

Table 3. MIC/MBC_{50} and MIC/MBC_{90} for MGB

Organism	MGB-BP-3 n=	MIC_{50} (mg/L)	MIC_{90} (mg/L)	MBC_{50} (mg/L)	MBC_{90} (mg/L)
Group B Streptococci	15	0.25	1	0.25	1
Group C Streptococci	15	0.25	1	0.5	1
Group G Streptococci	15	0.5	0.5	0.5	0.5
Methicillin-resistant *Staphylococcus aureus*	15	1	2	1	2
Methicillin-resistant *Staphylococcus epidermidis*	15	0.25	0.5	0.5	2
Methicillin-susceptible *Staphylococcus aureus*	15	0.5	1	1	2
Methicillin-susceptible *Staphylococcus epidermidis*	15	0.25	0.5	0.25	2
Streptococcus constellatus	15	0.25	0.5	0.5	1
Streptococcus mitis	15	0.5	2	0.5	2
Streptococcus pyogenes	15	0.25	0.5	0.25	2
Vancomycin-resistant *Enterococcus faecalis*	15	2	2	>32	>32
Vancomycin-resistant *Enterococcus faecium*	15	1	2	>32	>32
Vancomycin-susceptible *Enterococcus faecalis*	15	1	2	>32	>32
Vancomycin-susceptible *Enterococcus faecium*	15	1	2	>32	>32

In the Mutation Frequency study no MGB resistant mutants were observed with *Streptococcus pyogenes* or *Staphylococcus aureus*. However, MGB resistant mutants (up to 8x MIC) were observed with *Enterococcus faecalis* (Table 4).

Table 4. MIC data from the mutation frequency test for MGB against *Enterococcus faecalis*, *Staphylococcus aureus* and *Streptococcus pyogenes*..

Organism	MGB-BP-3 MIC (mg/L)		
	Broth microdilution	Agar Incorporation	Broth microdilution
Enterococcus faecalis	1	1	>64
Staphylococcus aureus	0.5	0.25	0.25
Streptococcus pyogenes	0.25	0.5	0.12

In the Serial Passage resistance study MGB showed a small MIC increase for *Enterococcus faecalis* from 0.25 to 8 mg/L in 4 days and for *Staphylococcus aureus* from 0.06 to 1 mg/L in 6 days. The MIC then remained unchanged throughout the rest of the passage. No increase in MIC was observed with *Streptococcus pyogenes*. In contrast, fusidic acid showed a pronounced increase in MIC over the 14 day passage (Figure 1).

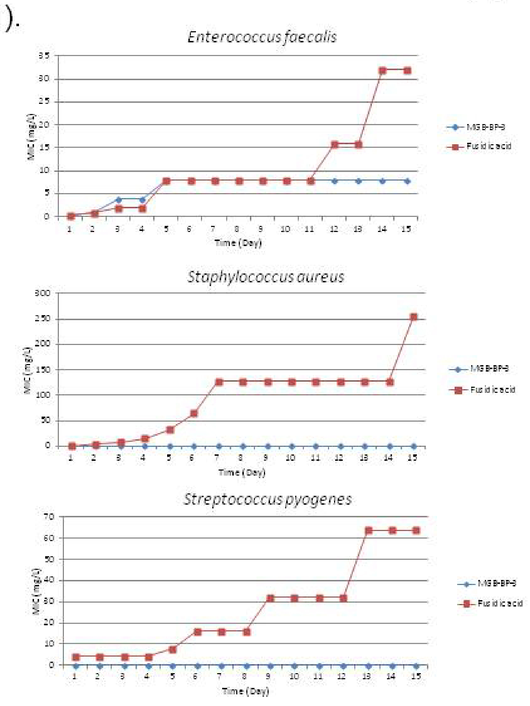

Figure 1. Serial passage with MGB and fusidic acid over 14 days with *Enterococcus faecalis*, *Staphylococcus aureus* and *Streptococcus pyogenes*.

CONCLUSIONS

- MGB has been shown to have strong inhibitory activity against all tested Gram-positive bacteria including vancomycin and meticillin resistant strains.
- Its activity against staphylococci and streptococci is bactericidal, and bacteriostatic against enterococci.
- The possibility of developing resistance to MGB was confirmed with *E. faecalis* but not with *S. aureus* and *S. pyogenes*

REFERENCES

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