B-1196b

MGB-BP-3, a New Class Antibacterial Agent, Effective in the Treatment of Severe *Clostridium difficile* Associated Diarrhoea in a Hamster CDAD Model

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ABSTRACT

Background: Vancomycin (Van) and metronidazole are effective in the treatment of CDAD, but their use is associated with a high incidence of relapse, so new therapies are needed. MGB-BP-3 (MGB) is a new class of antibacterial agent that binds selectively to the bacterial DNA Minor Groove and possesses strong in vitro bactericidal activity against Gm +ve- bacteria, including MRSA, VRE and *C. difficile*. We compared MGB and Van for efficacy in treating clindamycin-induced C. difficile infection in a hamster CDAD model.

Methods: Hamsters were given 30 mg/kg oral clindamycin, followed 24 h later by *C. difficile* (strain BI1) by gavage. 24 h after *C. difficile* administration, the hamsters received oral vehicle, Van (25 mg/kg bid), MGB (10 mg bid) as a freebase or as colonic delivery microparticles for 5 days. Animals were observed for 7 days following therapy when they were euthanized . Study endpoints included hypothermia, clinical deterioration, diarrhoea, mortality and *C. difficile* burden in the ileum, caecum and colon.

Results: All vehicle treated animals succumbed to infection 32-36 h post infection. No MGB or Van treated animals showed signs of morbidity during the 5 day treatment. 3 days following end of treatment with MGB freebase hamsters started to deteriorate clinically. All hamsters treated with the MGB microparticle formulation and Van survived to the end of the study and remained healthy. The mean survival times of the treatment groups were 2, 11.2, > 13 and >13 days for the vehicle, MGB freebase, MGB microparticles, and Van respectively. Survival was superior to the vehicle for all treatment groups. *C. difficile* burden in the colon and caecum was much higher in animals treated with Van compared to MGB microparticles. There was no difference in the MICs before and after treatment, indicating that resistance did not develop during therapy.

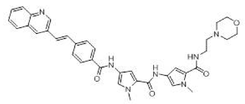
Conclusion: Our results indicate that MGB may be superior to Van for the treatment of CDAD.

INTRODUCTION

Vancomycin and metronidazole are effective in the treatment of *Clostridium difficile* Associated Diarrhoea (CDAD), but their use is associated with a high incidence of relapse. There is therefore a need for new therapies.

MGB-BP-3 is a new class antibacterial agent, under preclinical development at MGB Biopharma, it binds selectively to the Minor Groove of bacterial DNA and has strong *in vitro* bactericidal activity against Gram positive bacteria, including MRSA, VRE and *C. difficile*.

MGB-BP-3 was originally developed by researchers at the University of Strathclyde[1]. It has the architecture of a typical minor groove binder and is based upon the structure of a naturally occurring product, distamycin. The high activity against Gram positive bacteria and selectivity with respect to mammalian cells appear to be associated with the following features: (i) the quinoline ring and alkene (an amide isostere) lead to exceptionally strong DNA binding at AT rich sites, as shown by footprinting studies; (ii) the benzene ring reduces the inherent curvature compared with distamycin; and (iii), the N-ethylmorpholine tail group is associated with low mammalian cell toxicity.



MGB-BP-3

In this study we tested the inhibitory activity of two formulations of MGB-BP-3 against *C. difficile* isolates and examined their therapeutic effects on clindamycin-induced CDAD in hamsters. The efficacy of MGB-BP-3 in treating clindamycin-induced diarrhoea was compared to the effects of vancomycin in this animal model. We compared the rates of regrowth of *C. difficile* isolates in hamsters following discontinuation of MGB-BP-3 or vancomycin treatment, this being the most important indicator of potential relapse of CDI.

The study endpoints included hypothermia, clinical deterioration, diarrhoea, mortality and *C. difficile* burden in the ileum, caecum and colon.

METHODS

Material

MGB-BP-3 was prepared either as a freebase suspension or a special formulation for localised delivery of drugs to the ileo-colonic regions of the GI tract. Kuecept's ProRelease™ technology was used to load MGB-3-BP into delayed release microparticles using the cationic polymer Eudragit L100 and a target drug loading of 20 % w/w.

Hamster models of severe *C. difficile* induced diarrhoea

Clostridium difficile, REA-type BI1, were grown anaerobically on blood agar plates at 37°C for 3 days to induce sporulation. *C. difficile* spores were purified by exposure to ethanol and quantified before storage at -80°C. Spores were diluted in saline for orogastric inoculation into hamsters.

METHODS

Groups of 6 Golden Syrian hamsters (implanted with a temperature telemetry chip) weighing ~100g were preconditioned using 30mg/kg oral clindamycin 24h before infection[2]. Following preconditioning, the hamsters were infected by gavage with 100 spores and 400 vegetative *C. difficile* BI1.

Treatment was initiated 20h post infection and administered orally for 5 days. Hamsters were treated twice daily with 10mg of MGB-BP-3 formulated either as a freebase suspension or a suspension of microparticles, 25mg/kg BD vancomycin or a vehicle. Hamsters were observed for up to 13 days post infection and euthanized when severe infection developed (hypothermia <32°C, severe diarrhoea, immobile) or at the end of the observation period. Following euthanasia, sections of the small intestine, caecum and colon were removed and quantitative *C. difficile* cultures established.

In vitro MIC tests

MIC tests (MGB-BP-3 freebase and vancomycin) were performed against the *C. difficile* isolate before and after the infection model according to CLSI M11-A7[3] .

Statistical analysis

Survival times of hamsters were analysed using the statistical Kruskal-Wallis test (corrected for multiple comparisons) or the Mann Witney Test when appropriate.

RESULTS

In vitro dissolution studies showed that the MGB-BP-3 microparticles did not release or dissolve during the early 'acid phase' at pH 3.8 which mimicked the gastric conditions in the hamster stomach. Upon switching the pH to 6.8 to mimic the intestinal/colonic pH, > 95% drug dissolution was observed (Figure 1).

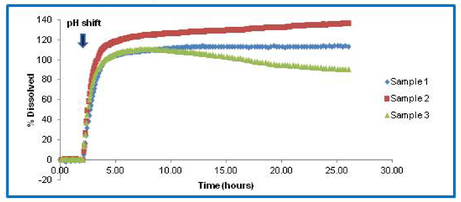


Fig. 1 - *In vitro* dissolution of the MGB-BP-3 microparticles

Following hamster infection, initial symptoms of wet tail were observed before 30 hours post infection. All untreated and vehicle treated hamsters succumbed to infection within 36 hours post infection, with symptoms including hypothermia, wet tail and severe diarrhoea.

All animals treated with MGB-BP-3 freebase suspension, MGB-BG-3 microparticle formulation and vancomycin survived throughout the treatment period.

3 days following the completion of treatment, hamsters that had been treated with MGB-BP-3 freebase deteriorated clinically, developing wet tail and hypothermia.

Hamsters treated with MGB-BP-3 microparticle formulation and vancomycin survived to the end of the study and remained healthy (Figure 2).

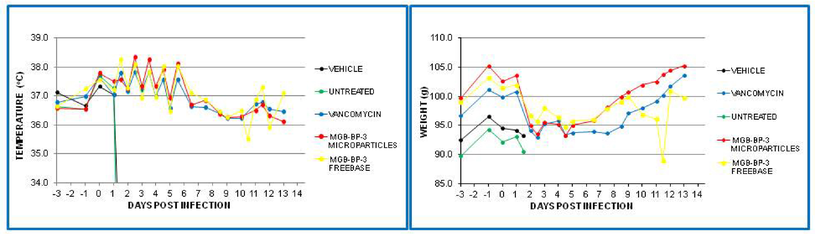


Fig. 2 – Temperature (left) and weights (right) of hamsters following *C. difficile* infection treatment

The mean survival times of the treatment groups were 2 days, 11.2 days, > 13 days and >13 days for the vehicle, MGB-BP-3 freebase, MGB-BP-3 microparticle and vancomycin respectively. Survival was very significantly superior to the vehicle for all treatment groups (Figure 3).

MGB-P-3 microparticle formulation treatment was the most effective treatment at reducing the burden of *C. difficile* in the small intestine, caecum and colon (Figure 4).

The MIC of MGB-BP-3 against *C. difficile* before and after infection showed no difference before and after treatment, indicating that resistance did not develop during the 5 day therapy (Table 1).

RESULTS

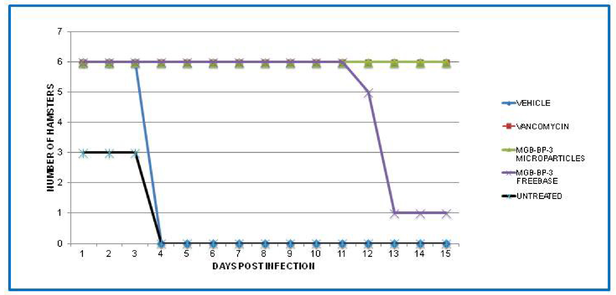


Fig. 3 - Survival of hamsters following *C. difficile* infection treatment .

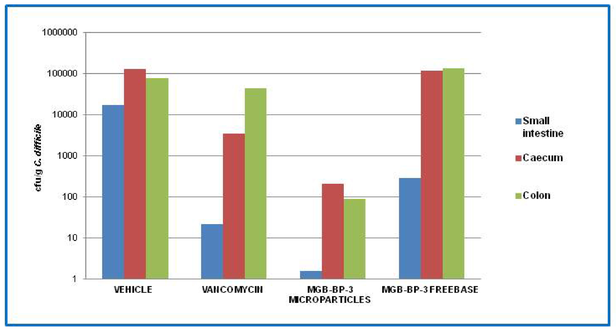


Fig. 4 – *C. difficile* burden in the small intestine, caecum and colon at the time of euthanasia.

Table 1 – MIC values of *C. difficile* before infection and after treatment

Strain identification	MGB-BP-3 (µg/mL)	Vancomycin (µg/mL)
Wild type BI1	0.03- 0.25	0.25
Vehicle treated hamsters	0.125-0.25	0.125-0.5
MGB-BP-3 freebase treated hamsters	0.25-1.0	0.25-0.5
MGB-BP-3 microparticle treated hamsters	0.25-1.0	0.25-0.5
Vancomycin treated hamsters	0.25-1.0	0.25-0.5

CONCLUSION

- MGB-BP-3 API and MGB-BP-3 microparticle formulation administered at 10mg/hamster BD was highly effective at improving survival in a hamster model of *C. difficile* associated diarrhoea.
- MGB-BP-3 microparticle formulation administered at 10mg/hamster BD was at least as effective at improving survival as 25mg/kg BD oral vancomycin in a hamster model of *C. difficile* associated diarrhoea.
- MGB-BP-3 microparticle formulation administered at 10mg/hamster BD was at least as effective (and numerically superior) at reducing the recovery of *C. difficile* from the small intestine, caecum and colon compared to 25mg/kg BD oral vancomycin in a hamster model of *C. difficile* associated diarrhoea.
- MIC tests did not detect a change in value following *in vivo* treatment, indicating that resistance did not develop during therapy.

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