P010

Evaluation of antifungal efficacy of two novel cyclic lipopeptides of the class *Bacillomycin* from *Bacillus subtilis* RLID 12.1 in a murine model of invasive candidiasis

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Objective: To evaluate the *in vivo* efficacy of HPLC–purified antifungal lipopeptides (AF4 and AF5) in a murine model of disseminated candidiasis.

Methods: *C. albicans* AMR16294 isolate was used for all the *in vivo* experiments. A total of 6-week-old pathogen-free, female BALB/c mice, weighing 20-25 g were used for all animal experiments. For Kaplan-Mier analysis, mice were rendered neutropenic by a loading dose of 200 mg/kg cyclophosphamide three days prior (D-3) to infection and 150 mg/kg (D + 1) maintenance dose on day 1 post-infection (D + 1). A total of 60 mice were randomized into 8 different groups with 5 or 6 animals in each group. Animals were infected with 100μL of $\sim 1 \times 10^5$ blastospores (corresponding to LD90) via the lateral tail vein. AF4 and AF5 were formulated in sterile PBS and administered intraperitoneally at doses of 5 mg/kg and 10 mg/kg body weight and compared with a clinically-relevant human equivalent dose of caspofungin. AF4, AF5, caspofungin, or vehicle were administered at 1 h and 24 h post-infection. The survival of the mice was monitored for 14 days post-infection. For organ fungal-burden assessment, mice from each group were euthanized by CO_2 inhalation, and the organs were aseptically removed, homogenized, and cultured on SDA.

Results: Both the doses of AF4 significantly reduced the mortality of mice compared to vehicle-treated mice. The survival over 2 weeks in 5 mg/kg, 10 mg/kg, and caspofungin arms were similar and no death was reported in the three groups (P <.01). In contrast, the mortality in-vehicle- administered group was 80% with a median survival of 8 days. A similar survival benefit was observed in AF5-treated mice. While the median survival in the vehicle-treated arm was 5 days, the 2-week survival in 5 mg/kg and 10 mg/kg arms was 80%-100%, comparable to that in the caspofungin arm (P <.01) (Fig. 1).

The median CFU/g kidney tissue in 5 mg/kg arm of AF4 was 1.3×10^4 equivalent to a 4-log reduction compared to the vehicle arm (3.8×10^8 CFU/g kidney, P <.0001). The *in vivo* efficacy was higher at a higher dose with the kidney homogenates of 10 mg/kg arm yielding sterile cultures comparable to that of CAS arm (Fig. 2). Similar organ fungal-burden reduction was noted in heart and splenic tissues with a median cfu/g tissue of 1.3×10^4 in 10 mg/kg, while CAS arms yielded sterile cultures.

In AF5 treated groups, the median cfu/g kidney tissue in 5 mg/kg arm was 1.3×10^4, however, the heart and splenic tissue homogenates yielded less fungal burden with median (q2) cfu/g as 0, while q3 of $6.7 \times 10^3$and 1.3×10^4, respectively (Fig. 2).

Conclusion: Both the antifungal compounds demonstrated a remarkable *in vivo* efficacy against *C. albicans* with a significant improvement in survival and a reduction in the organ-fungal burden.

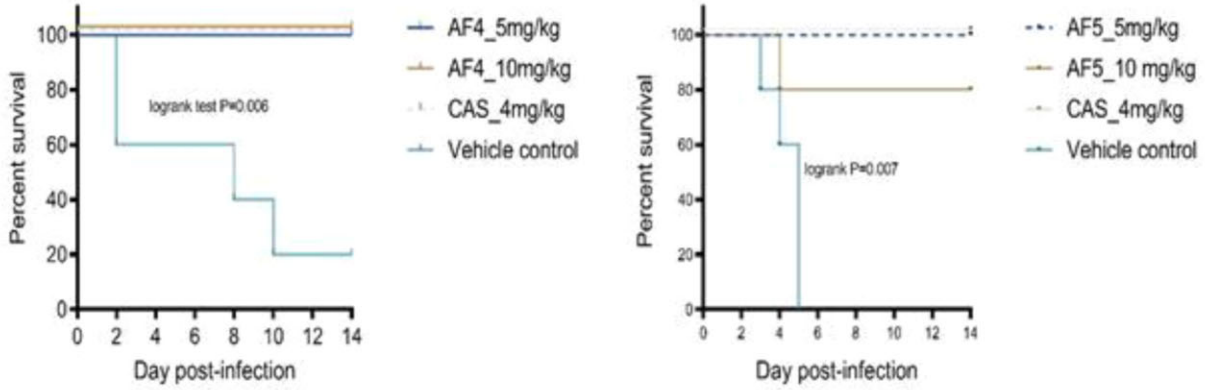

Figure 1. Kaplan-Meier survival curves demonstrating the *in vivo* efficacy of AF4 and AF5 in a neutropenic murine model of disseminated *C. albicans* infection

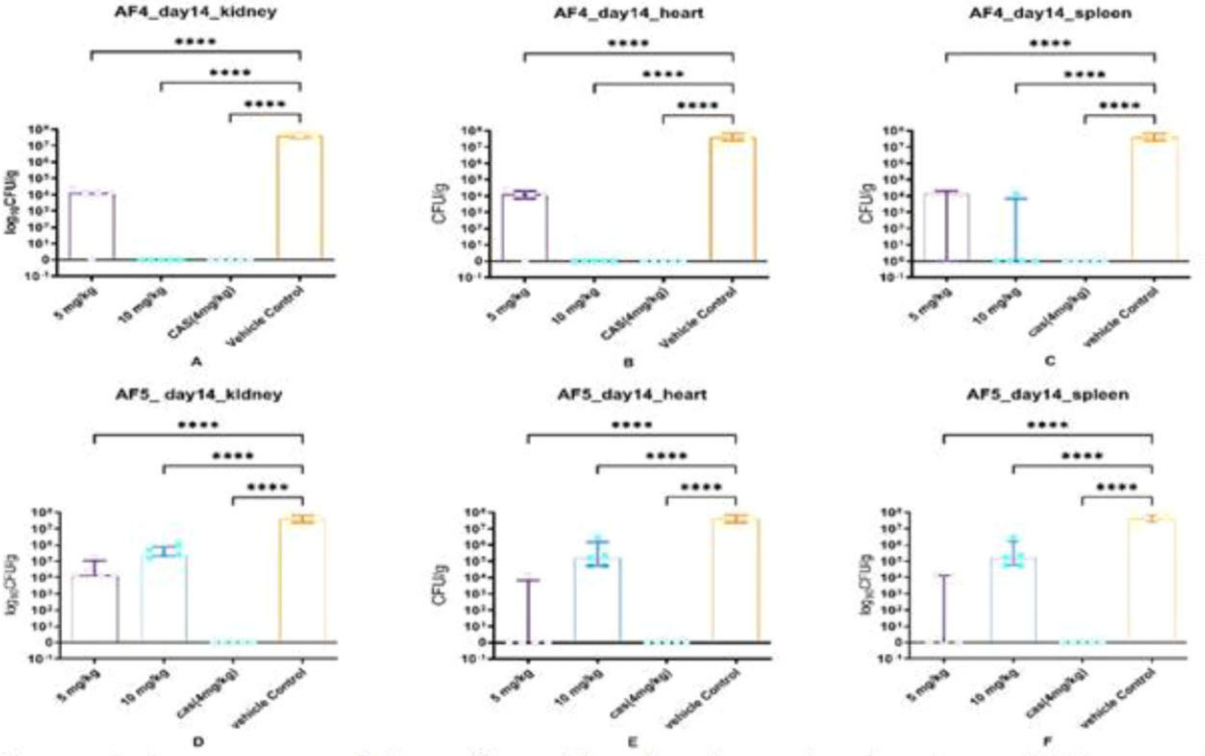

Figure 2. Assessment of tissue fungal-burden determined as $\log_{10}$ CFU/gram of kidney, spleen, and heart after administration of AF4 and AF5 at two doses each of 5 mg/kg and 10 mg/ kg body weight.