Original Article
BG40018: a promising drug candidate for the treatment of invasive fungal infections

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Abstract: Objective: BG40018 is a semisynthetic echinocandin derived from anidulafungin. This study was conducted to evaluate the pharmacokinetics and efficacy of BG40018. Methods: The \textit{in vitro} antifungal activities of BG40018 and anidulafungin were determined using CLSI broth microdilution methods and time-killing test. The pharmacokinetics of BG40018 and anidulafungin were compared in beagle dogs after 10-minute intravenous infusion at 1 mg/kg. Comparison of the \textit{in vivo} potency was studied in a murine systemic candidiasis model, the kidney fungal burden, pathological examination and survival rate were evaluated after treatment. Results: BG40018 showed comparable \textit{in vitro} antifungal activities to anidulafungin. Pharmacokinetics of BG40018 in the dogs resulted indicated that BG40018 exhibited longer elimination half-life and higher exposure compared to anidulafungin. BG40018 displayed more potent \textit{in vivo} efficacy against infections by reducing fungal burdens in kidney with improvement of pathological damage compared to anidulafungin. In addition, BG40018 demonstrated higher survival rate in a murine systemic candidiasis model than anidulafungin. Conclusions: All the data above support the characterization of BG40018 as a promising long-acting drug candidate for the treatment of serious, life-threatening, invasive fungal infections, and it will be investigated further for future clinical use.

Keywords: BG40018, echinocandin, long-acting, antifungal agents

Introduction

Opportunistic and invasive fungal infections have emerged as a major cause of hospital related morbidity and mortality in immunocompromised individuals such as HIV, cancer and organ transplant patients [1-4]. The incidence of fungal infections has increased at a staggering rate in the past few decades, and the reported mortality rates of systemic infection are greater than 30% [5-7].

The most common antifungals currently used for invasive fungal infections areazole antifungal agents and Amphotericin B. However, ongoing emergence of drug-resistance in primary, opportunistic fungal pathogens and problems of host toxicity have prompted the search for the development of new antifungal agents [8-12]. Echinocandins are lipopeptide antifungal agents that are potent noncompetitive inhibitors of (1, 3)-\(\beta\)-D-glucan synthase, an enzyme essential to the structural integrity of the fungal cell wall [13]. The absence of cell wall in mammals helps make the echinocandins very attractive in terms of low toxicity and reduced side effects. Also, the low incidence of resistance and few drug-drug interactions give the echinocandins advantages over other classes of antifungal drugs [14]. Echinocandins are now considered as important agents for treatment of serious fungal infections [15]. Anidulafungin, the most recently developed echinocandin, has fungicidal activity against a broad spectrum of \textit{Candida spp}, including those resistant to azoles and polyenes, and also has fungistatic activity against \textit{Aspergillus spp} [16, 17]. Nevertheless, anidulafungin is a poorly water-soluble semi-synthetic echinocandin, solubility enhancement using toxic surfactant such as Tween 80 for the formulation and the require-
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Various strategies to improve solubility and extend the elimination half-life were used in modifying anidulafungin. BG40018 is a semi-synthetic echinocandin derived from anidulafungin (Figure 1) with much better solubility, and exerts its antifungal activity via inhibition of fungal (1, 3)-β-D-glucan synthase. As a novel drug candidate of echinocandin, BG40018 confers both superior pharmacokinetics (PK) properties and water solubility. In this study, BG40018 demonstrated excellent in vitro and in vivo activity against fluconazole-sensitive and -resistant Candida species as well as Candida glabrata and Candida krusei. In addition, BG40018 exhibited longer elimination half-life and higher exposure compared to anidulafungin.

Materials and methods

Test articles

BG40018 and anidulafungin were semi-synthesized by BrightGene Bio-Medical Technology Co., Ltd. (Suzhou, China). Dosing solutions of BG40018 and anidulafungin for in vivo studies were prepared freshly by accurate weighing of compounds into appropriate sized containers and formulated in a vehicle consisting of 0.9% saline or 0.9% saline with 1% Tween 80, respectively.

Animals

C57BL/6 strain of female mice (6-8 weeks) were obtained from Shanghai Laboratory Animal Center of the Chinese Academy of Sciences. Male Beagle dogs (8~10 months) were purchased from Beijing Marshall Biotechnology Co., Ltd. All procedures performed on mice or dogs were approved by the Animal Care and Use Committee of facility.

Antifungal susceptibility testing

All strains were obtained from the American Type Culture Collection (ATCC). The MICs of BG40018 and anidulafungin were determined against each isolate according to the methods proposed by Clinical and Laboratory Standards Institute (M38-A2, M27-A3). BG40018 and anidulafungin were diluted two fold to the designated concentration range, and MICs were determined in Rosewell Park Memorial Institute (RPMI) 1640 buffered to a pH of 7.0 with MOPS. The starting inoculum was approximately 0.5×10³ to 2.5×10³ CFU/mL. Microtiter trays were incubated at 35°C in a moist, dark chamber, and the MICs were recorded after 24 h of incubation for all Candida spp. and after 72 h for C. neoformans. MIC values were defined as the lowest concentration of antifungal which inhibited 50% (MIC₅₀) or 90% (MIC₉₀) of visible growth.

Time-killing test

C. albicans SC5314, C. tropicalis ATCC20026, C. krusei ATCC2340, C. neoformans H99 were prepared at the starting inoculum of 10⁵ cells/mL. The concentrations were 2 μg/mL for BG40018 and anidulafungin. At predetermined time points (0, 4, 8, 12, 16 and 24 h) after incubation with agitation at 35°C, a 100 μL of ali-
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Table 1. In vitro activity of BG40018 and Anidulafungin against Candida species

<table>
<thead>
<tr>
<th>Organism</th>
<th>Antifungal agent</th>
<th>MIC (µg/mL)ᵃ</th>
<th>Range</th>
<th>50%</th>
<th>90%</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans</td>
<td>BG40018</td>
<td>0.0313-0.25</td>
<td>0.0625</td>
<td>0.125</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anidulafungin</td>
<td>0.0313-0.125</td>
<td>0.0313</td>
<td>0.0625</td>
<td></td>
</tr>
<tr>
<td>C. glabrata</td>
<td>BG40018</td>
<td>0.0625-0.5</td>
<td>0.125</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anidulafungin</td>
<td>0.0313-0.125</td>
<td>0.0313</td>
<td>0.0625</td>
<td></td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>BG40018</td>
<td>0.0625-0.25</td>
<td>0.0625</td>
<td>0.125</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anidulafungin</td>
<td>0.0625-0.125</td>
<td>0.0313</td>
<td>0.0125</td>
<td></td>
</tr>
<tr>
<td>C. kruseii</td>
<td>BG40018</td>
<td>0.125-1</td>
<td>0.125</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anidulafungin</td>
<td>0.0625-0.25</td>
<td>0.125</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>BG40018</td>
<td>0.0313-0.5</td>
<td>0.125</td>
<td>0.25</td>
<td></td>
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<tr>
<td></td>
<td>Anidulafungin</td>
<td>0.0313-0.125</td>
<td>0.0313</td>
<td>0.125</td>
<td></td>
</tr>
</tbody>
</table>

ᵃ50% and 90%, MIC₅₀ and MIC₉₀, respectively.

quot was removed from every solution and serially diluted 10-fold by sterile water. A 100 µL of aliquot from each dilution was spread on the sabouraud dextrose agar plate. Colony counts were determined after incubation at 35°C for 48 h.

Dog pharmacokinetics

The dogs received BG40018 or anidulafungin as a 10-minute intravenous infusion at 1 mg/kg. Whole blood samples (K₂ EDTA anticoagulant) were collected via jugular vein up to 72 h after dosing. The blood samples were centrifuged at 2000×g for 10 min at 4°C to obtain plasma, and plasma was stored below -70°C until analysis. Plasma samples were assayed for BG40018 or anidulafungin using liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods. BG40018 or anidulafungin from dog plasma samples were extracted using protein precipitation with acetonitrile followed by liquid–liquid extraction with deionized water. LC-MS/MS used an Agilent ZORBAX Eclipse XDB-C18 (50×2.1 mm, 3.5 µm) or SB-C18 (50×2.1 mm, 5 µm) column, operated at RT, at a flow rate of 0.4 mL/min with a gradient consisting of 0.1% formic acid in water and 0.1% formic acid in 95:5 acetonitrile/water coupled to an API 4000 (Applied Biosystems). The positive ionization mode with MRM transitions: m/z 1217.2→104.1 for BG40018, m/z 1140.7→343.3 for anidulafungin and m/z 494.2→369.1 for glibenclamide as internal standard (IS). Pharmacokinetic parameters were calculated from the plasma concentration-time data using standard noncompartmental methods and utilizing WinNonlin analysis software.

Kidney fungal burden and pathological examination

The mice were rendered neutropenic by injecting cyclophosphamide intraperitoneally (150 mg/kg) on 4 days and 1 day before infection. Disseminated infection with C. albicans SC5314 organisms were produced by injection of 1×10⁴ blastoconidia in 0.1 mL of saline via the lateral tail vein 2 h prior to drug therapy. BG40018 and anidulafungin were single administrated to the infected animals at 0.5, 1.5 and 4.5 mg/kg intravenously. The animals were euthanatized by CO₂ asphyxiation at 24 h post dose. After sacrifice, the kidneys of each mouse were immediately removed and placed in sterile water. The right kidney was homogenized in 0.5 mL PBS for fungal burdens measurement, and the left one was fixed in 10% neutral formalin for H&E (Hematoxylin and eosin) and PAS (Periodic acid-Schiff) staining. The homogenized kidney aliquots were plated onto SDA, and colony counts were performed after incubation for 48 h at 35°C.

Survival rate

The mice received cyclophosphamide intraperitoneally at 150 mg/kg on 4 days and 1 day prior to infection. A conidial suspension of 0.1 mL of 1×10⁴ conidia of C. albicans SC5314 was injected into lateral tail vein 2 h prior to drug therapy. The infectious doses were predetermined in preliminary experiments to produce high enough fungal burden and at the same time producing acceptably low lethality. BG40018 and anidulafungin were single administrated to the infected animals at 1.5 mg/kg intravenously, and control group was injected with normal saline. Survival status was monitored up to 25 days, survival rate and the median survival time were calculated.

Statistical analysis

Independent-samples T-test was used for pharmacokinetics parameter comparison. For kidney fungal burden test, Kruskal-Wallis nonparametric One-way ANOVA with Dunn’s post-test was used. Log rank test was applied to survival
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**Table 2. In vitro activity of BG40018 and Anidulafungin against C. neoformans and Aspergillus species**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Antifungal agent</th>
<th>MIC (µg/mL)a</th>
<th>Range</th>
<th>50%</th>
<th>90%</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. neoformans H99</td>
<td>BG40018</td>
<td>&gt;4</td>
<td>&gt;4</td>
<td>&gt;4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anidulafungin</td>
<td>&gt;8</td>
<td>&gt;8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspergillus fumigatus ATCC 13073</td>
<td>BG40018</td>
<td>0.125-8</td>
<td>2</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anidulafungin</td>
<td>0.125-16</td>
<td>4</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>

*a50% and 90%, MIC50 and MIC90, respectively.

**Figure 2. Time killing curves of**

C. albicans SC5314 (A), C. krusei ATCC2340 (B), C. tropicalis ATCC20026 (C), C. neoformans H99 (D) at the following concentrations of (●) Control, Anidulafungin 2 µg/mL (▼), BG40018 2 µg/mL (◇) for 24 h. Aliquots were obtained at the indicated time points and serially diluted were spread on agar plates. Colony counts were determined after 48 h incubation.

**Figure 3. Plasma Concentration-time Curves of BG40018 and Anidulafungin in Beagle Dogs.** Six dogs (two groups of three) received a single dose (1.0 mg/kg) of either BG40018 (◇) or anidulafungin (□) by 10-minute intravenous infusion. Each point on the graph represents a mean concentration of three dogs.

Results

**Antifungal susceptibility testing**

The MIC<sub>50</sub> and MIC<sub>90</sub> of BG40018 were ≤ 0.125 and 0.5 µg/mL for all tested Candida species, respectively (Table 1). BG40018 was approximately as active as anidulafungin with the MIC values within two gradients against all strains. C. neoformans and Aspergillus species were also tested, MIC values are presented in Table 2. Both of BG40018 and anidulafungin had less activity against C. neoformans and Aspergillus species.

**Time-killing test**

In addition, the fungicidal effect of BG40018 was confirmed by time-killing test (Figure 2). Against C. albicans SC5314, C. tropicalis ATCC20026 and C. krusei ATCC2340, both BG40018 and anidulafungin resulted in fungistatic activity. The tested agents resulting in approximately a 1-2 log10 CFU/mL decrease from the starting inoculum at 24 h. Compared to anidulafungin, BG40018 yielded approximately a 0.5-1 log10 CFU/mL decrease, and appeared to be slightly more active.

Against C. neoformans H99, both BG40018 and anidulafungin showed a modest growth inhibition activity. BG40018 also presented a slightly better activity than anidulafungin, yielding approximately a 0.3 log10 CFU/mL decrease.

**Dog pharmacokinetics**

The mean plasma concentration-time curves of BG40018 and anidulafungin in dogs are shown in Figure 3 and pharmacokinetic parameters are presented in Table 3. After administration by 10-minute intravenous infusion, BG40018
exhibited a slow clearance (0.208 mL/min/kg) and a long half-life (51 h), the AUC0-inf and Vdss were 80730 ng*h/mL and 0.794 L/kg, respectively. Anidulafungin exhibited a faster clearance (0.748 mL/min/kg) and a shorter half-life (17 h) compared to BG40018, the AUC0-inf and Vdss were 22396 ng*h/mL and 0.835 L/kg, respectively. These results indicated BG40018 exhibited longer elimination half-life and higher exposure compared to anidulafungin.

Kidney fungal burden and pathological examination

At 24 h post dose, BG40018 and anidulafungin was slightly effective at 0.5 mg/kg, while the kidney fungal burdens of mice intravenously infected with C. albicans SC5314 organisms significant decreased at 1.5 and 4.5 mg/kg. BG40018 treated mice had significantly lower fungal burdens in the kidneys as compared to those treated with anidulafungin at 1.5 and 4.5 mg/kg (p < 0.01; Figure 4A).

Moreover, H&E staining revealed that inflammatory infiltration and tissue necrosis of the kidneys from mice received BG40018 and anidulafungin treatment were significantly improved compared to mice without treatment. PAS staining also identified fewer hyphae in the kidneys of mice received BG40018 and anidulafungin treatment compared to without treatment. Furthermore, both H&E and PAS staining demonstrated the pathological damage of kidney from BG40018 treatment mice were slightly improved compared to anidulafungin treatment (Figure 4B).

Survival rate

All mice intravenously infected with C. albicans SC5314 organisms without treatment died within 3 days. By contrast, few mice treated with BG40018 or anidulafungin died within over a 25-day observation period. The mice treated with BG40018 had a much higher survival rate than those treated with anidulafungin. The median survival time of control group was 2 days, while BG40018 and anidulafungin group were ≥ 25 days (Figure 5).

Discussion

The in vitro susceptibility studies demonstrated potency of BG40018 against fluconazole-sensitive and -resistant Candida species as well as Candida glabrata and Candida krusei, variable but overall comparable to anidulafungin. The in vitro pharmacodynamics findings by time kill curves revealed that BG40018 fungicidal activity was similar with anidulafungin. Anidulafungin prevents fungal growth by inhibiting 1, 3-beta-D-glucan synthesis, an essential component of the cell wall of many fungi but absent in mammals. Anidulafungin does not rely on enzymatic degradation or hepatic or renal excretion, the drug is considered safe to use even in patients with any degree of hepatic or renal impairment [19]. However, intravenous injection the formulation of anidulafungin containing Tween 80 increase the risk of toxicity and/or irritability [20, 21]. The significantly improved solubility of BG40018 avoids use of toxic solvents. The long elimination half-life and robust in vitro antifungal activity make BG40018 a promising drug candidate that achieves higher plasma drug exposure with an extended interval dosing regimen compared to anidulafungin (Figure 3). To investigate the in vivo antifungal efficacy of BG40018, we assessed kidney burden reductions and survival rate post-antifungal treatment in mice infected with C. albicans. As expected, BG40018 displayed the potential efficacy against infections by reducing fungal burdens in kidney with improvement of pathological damage compared to anidulafungin (Figure 4). The superiority of BG40018 was mainly demonstrated by higher survival rate in mice infected with C. albicans compared to anidulafungin (Figure 5). These data are consistent with the susceptibility testing results and pharmacokinetics profile.

In conclusion, BG40018 is a novel, long-acting echinocandin drug that exhibits both in vitro and in vivo strong antifungal activities against fungal. Given its superior PK properties and

<p>| Table 3. Mean pharmacokinetic parameters of BG40018 and Anidulafungin in dogs |</p>
<table>
<thead>
<tr>
<th>PK parameters</th>
<th>Unit</th>
<th>BG40018</th>
<th>Anidulafungin</th>
</tr>
</thead>
<tbody>
<tr>
<td>t1/2</td>
<td>h</td>
<td>51±9.8**</td>
<td>17±6.3</td>
</tr>
<tr>
<td>AUC0-t</td>
<td>ng·h/mL</td>
<td>54917±6996**</td>
<td>21121±1404</td>
</tr>
<tr>
<td>AUC0-inf</td>
<td>ng·h/mL</td>
<td>80730±8938**</td>
<td>22396±1874</td>
</tr>
<tr>
<td>MRTIV</td>
<td>h</td>
<td>64±11**</td>
<td>19±5.3</td>
</tr>
<tr>
<td>CL</td>
<td>mL/kg/min</td>
<td>0.208±0.023**</td>
<td>0.748±0.062</td>
</tr>
<tr>
<td>Vdss</td>
<td>L/kg</td>
<td>0.794±0.148</td>
<td>0.835±0.207</td>
</tr>
</tbody>
</table>

**p < 0.01, (compare to anidulafungin, independent-samples T-test).
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Figure 4. Kidney tissue burden and Representative H&E (for the inflammatory cells influx and the extent of tissue necrosis) and PAS (for C. albicans) staining of temporarily neutropenic BALB/c mice infected intravenously with C. albicans SC5314 (infectious dose was 1×10^4 CFU/mouse). A. BG40018 was efficacious at single doses of 0.5, 1.5, and 4.5 mg/kg given intravenously at 24 h post dose, demonstrating dose dependent reductions of kidney burden that were greater than those of anidulafungin (AN) at the 1.5 and 4.5 mg/kg doses and similar to that of AN at the low dose (n=6 per group). Level of statistical significance is indicated at **p < 0.01 (Kruskal-Wallis nonparametric One-way ANOVA with Dunns post-test). B. H&E staining revealed that inflammatory infiltration and tissue necrosis of the kidneys from mice received BG40018 and anidulafungin treatment were significantly improved compared to mice without treatment. PAS staining also identified fewer hyphae in the kidneys of mice received BG40018 and anidulafungin treatment compared to without treatment.

Figure 5. Survival curves of C57BL/6 mice infected via the tail vein with 1×10^4 CFU C. albicans SC5314 (n=10 per group). Intravenous injection BG40018 (1.5 mg/kg), and anidulafungin (1.5 mg/kg) treatment was started 2 h after the infection. Infected animals were observed for over 25 days. BG40018 was more potent than anidulafungin at single dose of 1.5 mg/kg at 24 h after infection, **p < 0.01 (compare to anidulafungin, Log rank test).

solubility, BG40018 has the potential to be advantageous for dosing schedule and safety. All the data above support the characterization of BG40018 as a promising long-acting drug candidate for the treatment of serious, life-threatening, invasive fungal infections, and it will be investigated further for future clinical use.

Acknowledgements

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Disclosure of conflict of interest

None.

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