Efficacy of caspofungin, a 1,3-\(\beta\)-D-glucan synthase inhibitor, on \textit{Pneumocystis carinii} pneumonia in rats

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Abstract

\textit{Pneumocystis carinii} pneumonia (PcP) is a common and potentially fatal opportunistic infection in immunosuppressed patients, and the standard trimethoprim-sulfamethoxazole (TMP-SMZ) treatment has serious side effects. The cell wall of the causative fungal pathogen is enriched in 1-3-\(\beta\)-D-glucan, providing an alternative therapeutic target. We directly compared the efficacy of the 1,3-\(\beta\)-D-glucan synthase inhibitor caspofungin to TMP-SMZ for promoting survival and reducing lung cyst number during the early phase of treatment in a rat model of PcP. Rats were immunosuppressed using dexamethasone for 8 weeks and PcP infection confirmed in test animals by lung print smear. The remaining rats were randomly divided into three control groups, a baseline group and two observed for 7 or 14 days, two caspofungin groups treated intravenously for 7 or 14 days (1 mg/kg/d), and 2 TMP-SMZ positive control groups treated by oral gavage for 7 or 14 days (300 mg/kg/d). Mortality was markedly reduced by both caspofungin and TMP-SMZ after 14 days (caspofungin: 20.0\%, TMP-SMZ: 13.3\%, Control: 40.0\%). Body weight gain in caspofungin-treated rats after 7 (3.04 \(\pm\) 3.54\%) and 14 (4.27 \(\pm\) 2.79\%) days was similar to that in TMP-SMZ-treated rats (3.35 \(\pm\) 1.88\% and 5.85 \(\pm\) 2.78\%, respectively), whereas untreated controls showed weight loss. Lung weight to body weight ratio, and mean cyst number per 50 microscopic fields were significantly lower (all \(P<0.05\)) in caspofungin-treated rats than untreated controls at both 7 and 14 days, and similar to those in the TMP-SMZ-treated rats (all \(P>0.05\) vs. caspofungin). Caspofungin exhibited similar efficacy to TMP-SMZ for enhancing survival and reducing lung edema and cyst load in a rat model of PcP, suggesting potential clinical utility against PcP.

\textbf{Key words:} 1,3-\(\beta\)-D-glucan synthase inhibitor, \textit{Pneumocystis carinii} pneumonia, treatment.
Introduction

Pneumocystis carinii pneumonia (Pneumocystis pneumonia or simply PcP) remains the most prevalent and serious opportunistic infection in patients with acquired immunodeficiency syndrome [1]. It is also a common, devastating infection in patients with reduced immunity from other causes [2]. The number of human immunodeficiency virus (HIV)–negative patients with immune deficiency is rising with the widespread use of chronic immunosuppressive therapies, and those with PcP are prone to sudden-onset, rapidly progressive respiratory failure with high mortality (39%–60%) [2]. Furthermore, new at-risk patient populations are emerging, including patients with chronic obstructive pulmonary disease (COPD) [3] and those receiving anti–tumor necrosis factor therapy for autoimmune diseases [4,5]. The availability of a safe and effective treatment for PcP is thus of considerable clinical importance.

The first-line treatment for PcP is cotrimoxazole (TMP-SMZ), which is a combination of the antibiotics trimethoprim (TMP) and sulfamethoxazole (SMZ) [6]. These two agents are thought to act synergistically to inhibit tetrahydrofolic acid synthesis (and thereby DNA synthesis and transcription) in the causative pathogen Pneumocystis jirovecii by SMZ-mediated dihydropteroate synthetase and TMP-mediated dihydrofolate reductase inhibition [7]. However, TMP-SMZ is far from an ideal drug [8] as its use is associated with numerous adverse side effects including leucopenia, thrombocytopenia, and interstitial nephritis [9]. Unfortunately, the structure of P. jirovecii precludes the use of conventional antifungal agents as alternatives to TMP-SMZ.

Pneumocystis jirovecii is a yeast-like fungus [10] with cell walls that lack ergosterol, the target of antifungal azoles and polyenes [11]. Because the main component of the P. jirovecii cell wall is 1-3-β-D-glucan [12], agents such as echinocandins, which interrupt the synthesis of 1-3-β-D-glucan, have potential for treatment of PcP. Inhibition of 1-3-β-D-glucan synthase leads to destruction of the fungal cell wall, osmotic instability, and, ultimately, cell death [13]. In view of the limitations of current therapies for PcP, echinocandins such as caspofungin may represent the only effective options available for management of PcP [14]. Indeed, the fungicidal activity of caspofungin has already been demonstrated in diseases caused by Candida spp. and Aspergillus spp. [15]. An additional advantage of echinocandins is the low incidence of adverse events [16]. Despite evidence of safety, however, only a small number of case reports have described the use of caspofungin for management of PcP. Beltz et al. successfully used caspofungin and TMP-SMZ for the treatment of PcP in a child with acute lymphoblastic leukemia, with no reported adverse reactions [17]. Zhang et al. [18] and Mu et al. [19] also described successful treatment of PcP with caspofungin, and additional studies have concluded that caspofungin is an effective adjunct or salvage treatment for PcP [20–24]. For example, caspofungin cured P. jirovecii pneumonia in two solid organ transplant patients following failure of TMP-SMZ [24]. Moreover, caspofungin and other echinocandins reduced transmission of PcP in a mouse model [25]. However, low-dose caspofungin (0.1 mg/kg/d) failed to eradicate Pneumocystis in another mouse study [26], although caspofungin plus TMP-SMZ was superior to TMP-SMZ alone.

The present study was designed to provide a basis for possible future clinical trials by directly comparing the efficacy of caspofungin to that of TMP-SMZ in a rat PcP model.

Materials and methods

Drugs

Caspofungin acetae (>98% purity) was purchased from Merck, Sharp & Dohme (North Ryde, Australia; lot N1171), TMP-SMZ (>99% purity) from Tonghua Mao Pharmaceutical Co. Ltd (Tonghua, China; lot H22022741), dexamethasone sodium phosphate (>99% purity) from Fujian Sanai Pharmaceutical Co. Ltd (Fujian, China; lot H35020163), and tetracycline hydrochloride (>98% purity) from Ding Biotechnology Co. Ltd (Beijing, China; lot DH341).

Animals

The ethics committee of Capital Medical University, Beijing, China, approved the study. One hundred and twenty inbred male Wistar rats weighing 150–200 g were used for this study. Animals were housed under a 12 h light–12 h dark cycle with food and water available ad libitum. Ten rats were randomly assigned to a normal control group and 110 were subjected to dexamethasone-induced immunosuppression to promote natural infection by P. jirovecii from the environment. The immunosuppression group received inguinal subcutaneous injections of 10 mg/kg dexamethasone twice weekly for 8 weeks. Tetracycline (1 g/l) was added to the drinking water to minimize bacterial infections. After immunosuppressive treatment, two rats were sacrificed by exsanguination from the abdominal aorta under anesthesia to confirm the presence of acute PcP. Gomori methenamine silver (GMS) staining of lung print smears revealed the presence of 5–10 cysts per microscope field, confirming successful generation of a rat model of PcP [23–27].
Table 1. Allocation of rats with Pneumocystis pneumonia to the various treatment groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Treatment</th>
<th>Dose (mg/kg/d)</th>
<th>Observation/treatment duration (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>15</td>
<td>None</td>
<td>–</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>15</td>
<td>None</td>
<td>–</td>
<td>7</td>
</tr>
<tr>
<td>C</td>
<td>15</td>
<td>None</td>
<td>–</td>
<td>14</td>
</tr>
<tr>
<td>D</td>
<td>15</td>
<td>Caspofungin</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>E</td>
<td>15</td>
<td>Caspofungin</td>
<td>1</td>
<td>14</td>
</tr>
<tr>
<td>F</td>
<td>15</td>
<td>TMP-SMZ</td>
<td>300</td>
<td>7</td>
</tr>
<tr>
<td>G</td>
<td>15</td>
<td>TMP-SMZ</td>
<td>300</td>
<td>14</td>
</tr>
</tbody>
</table>

TMP-SMZ, trimethoprim-sulfamethoxazole.

Animal groups

The remaining rats were randomly divided into 7 groups of 15 (Table 1) in order to compare the effects of caspofungin (1 mg/kg/d) administered intravenously for either 7 or 14 days (groups D and E) to TMP-SMZ (300 mg/kg/d) administered by oral gavage for 7 or 14 days (groups F and G) and to the three control groups (A, B, and C) for which the same volume of normal saline was administered intravenously. Once the PcP model was established, rats in group A were sacrificed immediately, those in group B were sacrificed after 7 days (to match groups D and F), and those in group C were sacrificed after 14 days (to match groups E and G). Treatment duration was the time for which the animals were kept alive before they were sacrificed by exsanguination from the abdominal aorta under anesthesia for pathological examination.

Efficacy studies

All rats were examined for weight change, lung weight, and the ratio of lung weight to body weight (%). In addition, lung tissue impression smears were prepared from all five lung lobes. Briefly, lung tissue impression smears were air dried, fixed with methanol, and stained with GMS. Smears were then observed under a light microscope (BX51, Olympus Corp., Tokyo, Japan) to count the mean number of pneumocystis cysts per 50 randomly chosen microscope fields.

Statistical analysis

Statistical analysis was carried out using SPSS software (version 11.5; SPSS Inc., Chicago, IL, USA). Data are presented as the mean ± standard deviation. Group means were compared by analysis of variance and post hoc pair-wise Fisher least significant difference tests.

Results

Animal survival

At the end of the 14-day experimental period, all 10 rats in the untreated normal control group (no dexamethasone) survived. In contrast, immunosuppressed rats exhibited varying rates of mortality after 7 days (groups B, D, and F) and 14 days (groups C, E, and G; Table 2). Rats treated with either caspofungin or TMP-SMZ for 7 days (group D and group F, respectively) showed a higher rate of survival than those in control group B (groups D and F, 86.7% vs. group B, 66.7%). Similarly, 14-day survival was higher in caspofungin group E (80.0%) and TMP-SMZ group G (86.7%) than in control group C (60.0%).

Body weight, lung weight, and percentage of lung weight/body weight

Before initiation of treatment, there were no significant differences between groups in terms of body weight (P > 0.05). Body weight declined in untreated PcP rats as measured at both 7 days (group B, −5.34% ± 4.53%) and 14 days

Table 2. Mean survival, body weight, lung weight, and ratio of lung weight to body weight.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Survival (%)</th>
<th>Body weight before treatment (g)</th>
<th>Body weight after treatment (g)</th>
<th>Percentage body weight gain (%)</th>
<th>Lung weight (g)</th>
<th>Ratio of lung weight to body weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>15</td>
<td>100</td>
<td>225.07 ± 40.99</td>
<td>225.07 ± 40.99</td>
<td>0</td>
<td>2.30 ± 0.83</td>
<td>1.03 ± 0.32</td>
</tr>
<tr>
<td>B</td>
<td>10</td>
<td>66.67</td>
<td>215.90 ± 25.16</td>
<td>205.00 ± 23.15</td>
<td>−5.34 ± 4.53</td>
<td>2.36 ± 0.76</td>
<td>1.16 ± 0.39</td>
</tr>
<tr>
<td>C</td>
<td>9</td>
<td>60</td>
<td>226.78 ± 35.63</td>
<td>210.78 ± 39.04</td>
<td>−7.17 ± 5.25</td>
<td>2.50 ± 0.70</td>
<td>1.21 ± 0.35</td>
</tr>
<tr>
<td>D</td>
<td>13</td>
<td>86.67</td>
<td>202.85 ± 29.05</td>
<td>190.15 ± 27.48</td>
<td>3.04 ± 3.54</td>
<td>1.77 ± 0.54</td>
<td>0.84 ± 0.24</td>
</tr>
<tr>
<td>E</td>
<td>12</td>
<td>80</td>
<td>189.17 ± 22.40</td>
<td>197.42 ± 20.90</td>
<td>4.27 ± 2.79</td>
<td>1.59 ± 0.54</td>
<td>0.79 ± 0.23</td>
</tr>
<tr>
<td>F</td>
<td>13</td>
<td>86.67</td>
<td>213.00 ± 25.16</td>
<td>220.23 ± 24.30</td>
<td>3.35 ± 1.8</td>
<td>1.87 ± 0.45</td>
<td>0.86 ± 0.25</td>
</tr>
<tr>
<td>G</td>
<td>13</td>
<td>86.67</td>
<td>211.00 ± 25.95</td>
<td>221.38 ± 24.18</td>
<td>5.85 ± 2.78</td>
<td>1.71 ± 0.39</td>
<td>0.78 ± 0.22</td>
</tr>
</tbody>
</table>

aGroups are as follows: A, untreated, 0 d; B, untreated, 7 d; C, untreated, 14 d; D, caspofungin, 7 d; E, caspofungin, 14 d; F, trimethoprim-sulfamethoxazole, 7 d; G, trimethoprim-sulfamethoxazole, 14 d.
bP < 0.05 (for group D or F) compared with group B.
cP < 0.05 (for group E or G) compared with group C.
Table 3. Number of pneumocystis cysts.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean number of pneumocystis cysts</th>
<th>Reduction in cyst number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>15</td>
<td>609.07 ± 195.31</td>
<td>–</td>
</tr>
<tr>
<td>B</td>
<td>10</td>
<td>847.60 ± 294.01</td>
<td>39.16</td>
</tr>
<tr>
<td>C</td>
<td>9</td>
<td>1070.22 ± 372.86</td>
<td>75.71</td>
</tr>
<tr>
<td>D</td>
<td>13</td>
<td>49.92 ± 23.43b</td>
<td>91.80</td>
</tr>
<tr>
<td>E</td>
<td>12</td>
<td>30.69 ± 14.31c</td>
<td>94.96</td>
</tr>
<tr>
<td>F</td>
<td>13</td>
<td>72.23 ± 61.07b</td>
<td>88.14</td>
</tr>
<tr>
<td>G</td>
<td>13</td>
<td>27.08 ± 20.00c</td>
<td>95.55</td>
</tr>
</tbody>
</table>

*Groups are as follows: A, untreated, 0 d; B, untreated, 7 d; C, untreated, 14 d; D, caspofungin, 7 d; E, caspofungin, 14 d; F, trimethoprim-sulfamethoxazole, 7 d; G, trimethoprim-sulfamethoxazole, 14 d.

b P < 0.05 (for group D or F) compared with group B.

c P < 0.05 (for group E or G) compared with group C.

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(group C, −7.17% ± 5.25%). In contrast, body weight increased significantly (P < 0.05) in rats treated with either caspofungin or TMP-SMZ for 7 days (caspofungin group D, 3.04% ± 3.54%; TMP-SMZ group F, 3.35% ± 1.80%) and 14 days (caspofungin group E, 4.27% ± 2.79%; TMP-SMZ group G, 5.85% ± 2.78%). Body weight gain was similar in TMP-SMZ and caspofungin groups (P > 0.05). In addition, significant differences were observed between the treated and untreated groups in terms of the ration of lung weight to body weight. In the untreated PcP rats, values were >1.0% at 0, 7, and 14 days (groups A, B, and C) but significantly lower (<0.9%) in all caspofungin and TMP-SMZ treatment groups (P < 0.05; Table 2). These results likely reflect a reduction in pulmonary edema and formation of alveolar exudates in caspofungin- and TMP-SMZ–treated rats. Thus, disease progression was less severe in PcP rats treated with caspofungin or TMP-SMZ.

**Number of pneumocystis cysts**

No cysts were observed in lung print smears from normal control rats that were not administered dexamethasone, whereas a substantial number of cysts were observed in the PcP groups (Table 3; Fig. 1). In the untreated PcP rats (groups A, B, and C), there was a progressive increase in the number of cysts per 50 microscope fields, from 609 ± 195 at day 0 to 1070 ± 373 at day 14. The number of cysts per 50 microscope fields was significantly lower (P < 0.05) in caspofungin-treated rats at both 7 days (50 ± 23) and 14 days (72 ± 61) and similar (P > 0.05) to cyst counts from TMP-SMZ groups. Thus, both caspofungin and TMP-SMZ dramatically reduced the number of pneumocystis cysts in the lungs of rats with PcP, and the efficacy of caspofungin was similar to that of TMP-SMZ.

**Discussion**

Although TMP-SMZ has been used as the first-line PcP treatment for many years, the toxicity and safety profile of this drug combination are far from ideal [8,9]. The identification of alternative therapies with equal or better efficacy and minimal adverse effects would undoubtedly improve the management of PcP. Inhibitors of 1-3-β-D-glucan synthase are promising alternatives to TMP-SMZ because they target a major component of the cell wall of the causative pathogen and have a superior safety profile [13,16]. Indeed, caspofungin reduced mortality, prevented body weight loss and lung weight gain, and inhibited the development of pulmonary pneumocystis cysts as effectively as TMP-SMZ in a rat model of PcP. Caspofungin may thus be a useful drug for the clinical management of PcP, and this issue warrants clinical trials.

Previous studies have shown that echinocandins effectively reduce lung cyst burden in animal models of PcP [25] and that caspofungin enhances the efficacy of TMP-SMZ [26]. Furthermore, Schmatz et al. [28] found that...
treatment with the 1-3-β-D-glucan synthase inhibitor L-671,329 (0.3 mg/kg) for 4 days resulted in a 98.1% reduction in pneumocystis cysts in rats, which is markedly higher than the 61.1% reduction observed with TMP-SMZ at the time point. Powles et al. [29] found that therapy with TMP-SMZ reduced pneumocystis cysts by 90% but required a longer treatment duration to achieve the same magnitude of reduction as caspofungin. We did not examine cyst burden after only 4 days of treatment, but caspofungin (1 mg/kg/d) was as effective as TMP-SMZ in reducing cyst burden, lung weight, and mortality after 7 and 14 days of treatment. In our study, rats were administered TMP-SMZ by gavage to ensure adequate dose control, while previous studies added it to the drinking water, making dose dependent on thirst. Given this uncertainty, it is unclear whether caspofungin is truly superior to TMP-SMZ. The most parsimonious conclusion is that caspofungin is as effective as TMP-SMZ at a much lower dose and may present a better safety profile. Adverse reactions are known to occur in approximately 7% of non-HIV individuals on TMP-SMZ and in up to 50% of HIV-infected patients; furthermore, some patients are sulfonamide intolerant [30]. The availability of alternative drugs such as caspofungin and other echinocandins will therefore enable better management of PcP in patient populations less responsive to TMP-SMZ.

In the present study, we administered caspofungin at 1 mg/kg/d, a dosage extrapolated from humans studies (70 or 50 mg/d) but different from those used in previous animal investigations [28,29]. While neither caspofungin nor TMP-SMZ exhibited obvious toxicity in PcP model rats, additional animal and clinical investigations are warranted in order to determine the side-effects profiles at different dosages and define the optimal range for safe and effective management of PcP.

We conclude that caspofungin acetate is as effective as TMP-SMZ for the treatment of PcP in rats. However, neither drug completely eliminated lung cysts after 14 days, so future studies are required to compare efficacies during longer treatment regimens.

Declaration of interest
The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

References


