Echinocandins for Pneumocystis jirovecii pneumonia in non-HIV patients: A case report

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Abstract. For the past two decades, echinocandins have shown prophylactic and therapeutic efficacy in patients with Pneumocystis jirovecii pneumonia (PJP), due to their ability to inhibit the synthesis of β-1, 3-glucan, a major component of the cell wall of P. jirovecii. The present study reported two cases of human immunodeficiency virus (HIV)-negative patients who received echinocandins as a salvage therapy at Peking Union Medical College Hospital (Beijing, China), both of whom exhibited good responses to treatment. In both cases, polymerase chain reaction of sputum or bronchoalveolar lavage specimens became negative following treatment. The present study also performed a literature search to identify non-HIV patients with PJP who previously received echinocandins. The results of the present study suggested that echinocandins maybe promising therapeutic agents in the treatment of non-HIV patients with PJP, particularly in combination with trimethoprim-sulfamethoxazole. Therefore, the results warrant a randomized controlled trial.

Introduction

As an opportunistic infection, Pneumocystis jirovecii pneumonia (PJP) is a severe and life-threatening complication experienced by immunocompromised patients (1). With the advent of highly active antiretroviral therapy and prophylaxis strategies, the incidence of PJP has decreased markedly among patients with human immunodeficiency virus (HIV), with a mortality rate <10% (2). By contrast, non-HIV patients with PJP are characterized by advanced age, increased comorbidities, non-classical clinical symptoms, rapid deterioration and poor prognosis (3-7). Once acute respiratory failure (ARF) develops, non-HIV patients with PJP most likely require intensive care and ventilatory support and have a mortality rate of up to 75.6% (5). Despite the aforementioned differences, treatment regimens remain the same for HIV and non-HIV patients with PJP; for example, the administration of trimethoprim/sulfamethoxazole (TMP-SMZ) as the first-line drug, then an adjunctive steroid (standard dosage) for severe ARF, followed by the second-line drug (8-15). Therefore, other treatment options need to be investigated.

Due to the ability to inhibit the synthesis of β-1, 3-glucan, a major component of the cell wall of P. jirovecii, echinocandins could be used in the treatment of PJP (15-20). During the past two decades, the prophylactic and therapeutic efficacies of echinocandins have been investigated in animal models and clinical studies (21,22). A cohort study demonstrated a high success rate (8/10) with caspofungin salvage treatment in HIV-patients with PJP; however, data regarding the treatment of PJP with echinocandins in non-HIV patients remain limited and controversial (15,20).

The current report described two HIV-negative cases of PJP in which echinocandins were used for salvage treatment at the Medical Intensive Care Unit, Peking Union Medical College Hospital (Beijing, China). Both patients provided written informed consent. A review of previous reports involving the use of echinocandins for the treatment of PJP in non-HIV patients was also conducted.

Case report

Case 1. A 71-year-old male presented to the Emergency Department of the Peking Union Medical College Hospital
Administration of immunosuppressive agents to the patient was discontinued. Despite this treatment, the patient remained febrile and exhibited increasing dyspnea requiring a reservoir face mask. Given the severity of the condition, the patient was transferred to the MICU to start high-flow nasal cannula (HFNC) oxygen therapy (flow of 60 l/min and 60% FiO2) on day 16; caspofungin (Merck & Co., Inc.) was administered as salvage therapy at a loading dose of 70 mg, followed by a maintenance dose of 50 mg daily. After 4 days of treatment with caspofungin, fever alleviated, respiratory conditions improved and the HFNC was replaced by conventional nasal prongs. The patient was transferred to the ICU ward on day 7. A follow-up chest CT scan demonstrated a partially normal appearance of the lung fields (Fig. 1D).

Quantitative polymerase chain reaction (qPCR). DNA was extracted using a commercial kit (QIAamp DNA Mini kit; Qiagen GmbH, Hilden, Germany) following the manufacturer's protocol, but with elution using 30 µl Tris-EDTA buffer solution (Beijing Leagene Biotech Co., Ltd., Beijing, China). Purified DNA was then used as a template to amplify the part of the mitochondrial gene, which encodes the large subunit of rRNA. PCR was performed using the following primers: pAZJ02-E forward, 5'-GATGCGTGTGTTCAAGCCCA-3' and reverse, 5'-GTGTACGTGCAAGTACTC-3'. Albumin forward, 5'-TGGTGAAATGGCTGACTGCT-3' and reverse, 5'-CTC TGGTCTCACCATCGGG-3'. PCR was performed with a total reaction volume of 20 µl, comprising: Template DNA 2 µl (10-100 ng), 0.4 µM of forward primer 0.4 µl, 0.4 Mm of reverse primer 0.4 µl, ddH2O 6.8 µl and the SYBR Green Mix (2x) 10 µl (SYBR Green qPCR Master Mix, MedChemExpress USA, Monmouth Junction, NJ, USA). The thermocycling conditions were as follows: Pre-amplification denaturation at 95°C for 5 min; 40 cycles of 95°C for 15 sec, 60°C for 30 sec and 72°C for 30 sec. The relative amount of gene expression was normalized using Albumin and was calculated using the 2−ΔΔCq formula as previously described (23). PCR mixtures were prepared in a laminar-flow cabinet and several controls were implemented. All amplifications were performed in parallel with a negative control (ultrapure distilled water) and a positive control (BAL samples of patients with definite Pneumocystis pneumonia).

Gomorimethenamine silver staining. Slides were prepared using cytocentrifugation (13,800 x g, 15 min, 4°C), cut to a thickness of 2-3 µm and microwaved in a 10% chromic acid (Hubei XinRunde Chemical Co., Ltd., Hubei, China) solution at 65°C for 40 sec. Samples were then washed with water and cleared using 1% sodium metabisulfite for 30 sec. After the slides were washed with distilled water, they were placed in a Coplin jar containing 50 ml of methenamine working solution and microwaved at 65°C for a further 65 sec. The slides were rinsed again with distilled water and treated with 1% gold chloride for 2-5 sec. Following further rinsing with distilled water, samples were exposed to 5% sodium thiosulfate for 1 min, counterstained with a light green working solution (Beijing Leagene Biotech Co., Ltd.) and cleared using xylene. Samples were then covered with cover slips and examined using routine light microscopy at a magnification of x70.

Through a literature review, the present study identified 22 HIV-negative patients with PJP treated with
Echinocandins (8-20). The demographic and clinical characteristics of these cases, and the two patients included in the present study, are summarized in Table I. Among these patients, the mean age was 49.8 years. Underlying conditions varied between the included cases, with solid organ transplant most commonly reported (11/24; 45.8%). Only three patients were given primary prophylaxis. All enrolled cases exhibited concurrent bilateral pulmonary infiltrations and were clinically treated for pneumonia. *P. jirovecii* was the predominant pathogen that was confirmed by methenamine silver staining or PCR. Of all patients, 8 and 16 were treated with echinocandins as initial and salvage regimens, respectively, and the mean duration of treatment with echinocandins was 19 days. All included patients but one (11) were treated with caspofungin at 70 mg on the first day with a maintenance dose of 50 mg/day. Associations between the type of echinocandin treatment strategy, adjunctive corticosteroids and the underlying disease are described in Table II.

**Discussion**

The present study described two successful cases of treatment with echinocandins for non-HIV patients with PJP. In addition to the two cases in the present study, 24 non-HIV PJP cases treated with echinocandins were identified in the literature (8-20). This revealed that, although a rationale exists for the use of echinocandins in the treatment of PJP, the current clinical use appears to be low. Potential reasons for this may include that fact that the use of echinocandins to treat PJP has only appeared in recent years, the lack of convincing efficacy data and that the use of echinocandins for PJP treatment is currently off-label (11,19,20).

The present study analyzed the application strategy of caspofungin in non-HIV patients with PJP. The results reported in previous studies suggested that echinocandins were most frequently considered as a salvage regimen (16/24), with a mortality rate of 31.5% (5/16 patients) (8-9,11,13-16,19,20). As of yet, no sufficient data for the outcome of echinocandins as a salvage regimen in non-HIV patients with PJP have been reported. In fact, for HIV patients who fail to respond to initial treatment, several traditional salvage regimens have shown high therapeutic effectiveness, including the combination of clindamycin and primaquine [42-44/48 (88-92%)] or atovaquone [4/5 (80%)] (24). However, regarding non-HIV patients with PJP, the data of these traditional salvage regimens is limited. In a previous study, clindamycin-primaquine or pentamidine were used as salvage regimens following treatment failure of the first-line regimen in 12 non-HIV patients with PJP and the mortality rate was 66.7% (8/12) (25). Therefore, the present results indicated that use of echinocandins (caspofungin starting with a loading dosage of 70 mg followed by 50 mg/day) as salvage therapy resulted in favorable and comparative rates of mortality in non-HIV patients compared with previous salvage regimens; however a statistical comparison was not possible. This suggests that an echinocandin-based salvage regimen maybe an option for the treatment of PJP in non-HIV patients.

There were 8 cases from previously published studies that used an echinocandin-based regimen for the initial
Table I. Clinical characteristics of the reported cases of echinocandins use for *Pneumocystis jirovecii* pneumonia in patients.

| Author, year | Patient no | Age/ gender | Underlying Disease | Initial treatment | Cause of EC use | Salvage regimen | Time to use EC (days) | Steroid used? | Duration of EC (days) | End (Refs.) |
|--------------|------------|-------------|--------------------|-------------------|-----------------|-----------------|----------------------|--------------|----------------------|-------------|
| Zhang et al., 2006 | 1 | 93/M | COPD | TMP-SMZ | Adverse reaction | CA | 32 | No | 42 | S (8) |
| Annaloro et al., 2006 | 2 | 45/M | For HSCT | TMP-SMZ | Treatment failure | CA+ TMP-SMZ | 11 | Yes | 45 | S (9) |
| Belz et al., 2006 | 3 | 5/M | ALL | CA+ TMP-SMZ | Empirical use | None used | NR | Yes | 22 | S (10) |
| Kamboj et al., 2006 | 4 | 13/M | HSCT | CA | Antifungal | Pentamidine | NR | No | 18 | D (11) |
| Kamboj et al., 2006 | 5 | 42/F | ALL | TMP-SMZ | Antifungal | MI + Others | 9 | No | 30 | D (11) |
| Takeda et al., 2009 | 6 | 47/M | Liver TP | MI+ TMP-SMZ | Empirical use | MI+ TMP-SMZ | NR | No | N/A | S (12) |
| Zhang et al., 2012 | 7 | 58/F | Lung Cancer | N/A | Treatment failure | CA+ TMP-SMZ + CLI+PRI | NR | Yes | 33 | S (14) |
| Li, 2016 | 8 | 46/M | CKD | TMP-SMZ | Adverse reaction | CA+CLI | NR | Yes | 26 | D (15) |
| Kim et al., 2013 | 9 | 1/M | SCID | TMP-SMZ | Treatment failure | CA+ TMP-SMZ +AT+PRO (CLI+PRI) | 26 | No | 26 | D (15) |
| Kim et al., 2013 | 10 | 63/M | Liver TP | TMP-SMZ | Treatment failure | CA+ TMP-SMZ | 9 | No | 5 | D (15) |
| Kim et al., 2013 | 11 | 57/M | Kidney TP | TMP-SMZ | Treatment failure | TMP-SMZ + PRI+CLI (CA) | 18 | No | 11 | D (15) |
| Kim et al., 2013 | 12 | 46/F | Liver TP | TMP-SMZ | Treatment failure | CA+ TMP-SMZ (CLI+PRI) | 2 | No | 7 | S (15) |
| Tu et al., 2013 | 13 | 61/M | Kidney TP | TMP-SMZ | Adverse reaction | CA+ TMP-SMZ | >10 | Yes | 14 | D (16) |
| Tu et al., 2013 | 14 | 35/M | Kidney TP | TMP-SMZ | Adverse reaction | CA+ TMP-SMZ | 10 | Yes | 14 | S (16) |
| Tu et al., 2013 | 15 | 43/M | Kidney TP | CA+ TMP-SMZ | Empirical use | None used | 7 | No | 14 | S (16) |
| Jiang et al., 2013 | 16 | 46/M | LBC-L | CA | Adverse reaction | None used | 5 | No | NA | S (17) |
| Mu et al., 2009 | 17 | 76/M | CKD | CA | Adverse reaction | CA+ TMP-SMZ | 9 | Yes | 21 | S (18) |
| Hof and Schnüll, 2013 | 18 | 60/M | CML | TMP-SMZ | Treatment failure | CA | 9 | No | 21 | S (19) |
| Utili et al., 2007 | 19 | 57/F | Kidney TP | CA | Antifungal | CA+ TMP-SMZ | 1 | No | 14 | S (20) |
| Utili et al., 2007 | 20 | 28/M | Kidney TP | TMP-SMZ | Treatment failure | CA+ TMP-SMZ | 7 | Yes | 16 | S (20) |
| Utili et al., 2007 | 21 | 59/M | Heart TP | TMP-SMZ | Treatment failure | CA+ TMP-SMZ | 6 | Yes | 7 | S (20) |
| Utili et al., 2007 | 22 | 58/F | Heart TP | CA+ TMP-SMZ | Empirical use | None used | 1 | Yes | 14 | S (20) |
| Present study | 23 | 71/M | IgG4 | TMP-SMZ | Treatment failure | CA+ TMP-SMZ | 14 | Yes | 21 | S |
| Present study | 24 | 68/F | SLE | TMP-SMZ | Treatment failure | CA+ TMP-SMZ | NR | Yes | 7 | - |

ALL, acute lymphocytic leukaemia; AT, atovaquone; CA, caspofungin; CKD, chronic kidney dysfunction; COPD, chronic obstructive pulmonary disease; CLI, clindamycin; CML, chronic myelocytic leukaemia; D, mortality; DM, diagnosis method; EC, echinocandins; F, female; HSCT, hematopoietic stem cell transplant; LBC-L, large-B-cell lymphoma; M, male; MI, micafungin; N/A, not available; NR, no report; PRO, proguanil; PRI, primaquine; Pt, patient; Refs., reference; S, survived; SCID, severe combined immune deficiency; TP, transplant.
treatment (10-12,16-18,20) and exhibited a good response with survival rates of 87.5%. When only the 6/8 cases that used the combination regimen of echinocandins and TMP-SMZ were considered, an excellent response was observed as all 6 patients had survived the infection. When the cases that used the combined regimen of echinocandins and TMP-SMZ in salvage therapy were also included, the mortality rate was 13.3% (2/15). These results appeared to favor the combination of echinocandins and TMP-SMZ.

The combined regimen of echinocandins and TMP-SMZ has been associated with high effectiveness in clearing the invading P. jirovecii (26). P. jirovecii is a fungus that exists in either trophic or cyst forms (trophic/cyst 9:1) (27). The primary component of the cyst cell wall is β-1,3-glucan, which is poorly expressed in the trophic forms. Therefore, echinocandins mainly act on cyst forms by inhibiting the β-1,3-glucan synthase enzyme and disturbing the integrity of the cell wall (21,22). Previous experiments suggested that the use of caspofungin alone was associated with a 90% decrease in cyst forms after 4 days and a 66% reduction in trophic forms after 21 days of treatment. Furthermore, in an animal model of PJP, the administration of low doses of caspofungin with TMP-SMX may provide an improved clearance of Pneumocystis infection (28). On the other hand, echinocandins exhibit reduced activity against the trophic forms of P. jirovecii and the use of echinocandin alone may not completely eradicate the infection. Therefore, the co-administration of echinocandins and TMP/SMZ, which is primarily active against trophic forms, may exert synergistic activity against P. jirovecii by fully inhibiting the life cycle of the organism. However, clinical data regarding this combined therapy regimen were confined to case reports only (13,16,25).

In conclusion, positive clinical effects of echinocandins were observed in the present study, which suggested that the addition of echinocandins to TMP/SMZ was active against trophic forms and may demonstrate activity against P. jirovecii by inhibiting the organism's life cycle. However, the present analysis was based exclusively on case reports. It is possible that patients with favorable outcomes from the use of echinocandins may have been selectively reported in these case reports. Therefore, a large sample, well-designed randomized trials, particularly in the use of echinocandins combined with TMP-SMZ for the treatment of PJP are warranted to verify these results.

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All data generated or analyzed during this study are included in this published article.

Authors' contributions
HBH, JMP and BD contributed to the conception, design and data interpretation. All authors read and approved the manuscript.
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Consent for publication
Patients provided written informed consent to participate.

Competing interests
The authors declare that they have no competing interests.

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