Caspofungin in the treatment of candidosis and aspergillosis

Johan Maertens(1) and Marc Boogaerts(1)

Antifungal agents can be classified by their site of action in fungal cells, which can have important implications for both efficacy and tolerability. Currently available agents include the polyenes, nucleoside analogs, and the azoles. With the exception of 5-fluorocytosine, all agents act by interfering with the structural or functional integrity of the fungal plasma membrane. However, the non-selective nature of this therapeutic target results in concomitant cross-inhibition (or toxicity) in mammalian cells. New compounds that interfere with the fungal cell wall—a target not present in mammalian cells—therefore constitute an important focus of current clinical research. Caspofungin, the first representative of a new class of antifungals that inhibit β-(1,3)-D-glucan synthesis, exerts potent activity against Candida and Aspergillus spp. and appears to be generally well tolerated. This paper reviews the data on caspofungin.


INTRODUCTION

The incidence of invasive fungal infections has increased dramatically over the last two decades, due in large part to an increased number of immunocompromised patients, and increased use of invasive procedures.1 Mycoses with emerging pathogens such as Aspergillus and Candida spp. have become important causes of morbidity and mortality. The unacceptable high crude mortality rate of these infections—up to 90% for invasive aspergillosis—results in part from difficulties in obtaining a reliable diagnosis at an early stage of the disease.2-6 Hence, generalized prophylaxis and empirical antifungal therapy have been introduced in an effort to overcome the diagnostic obstacles, especially since the early start of adequate therapy appears to be crucial in improving the detrimental outcome of established disease.7 The implementation of sensitive, new diagnostic tools with good predictive value, such as galactomannan antigen detection, high-resolution pulmonary CT scanning, and detection of fungal DNA or metabolites, may allow a more targeted preemptive approach, directed towards true high-risk patients.8-10

The high mortality rate of these infections results also from shortcomings in the available therapeutic arsenal.11 Amphotericin B deoxycholate, flucytosine and itraconazole are associated with low success rates, and are hampered by serious infusion- or drug-related toxicity, by hazardous drug interactions, by pharmacokinetic problems, and by the development of resistance.12 Fluconazole is devoid of any activity against Aspergillus spp., and its widespread (prophylactic) use has resulted in increasing reports of treatment failure and resistant Candida organisms, most notably—but not exclusively—in HIV-infected patients.13 Lipid formulations of amphotericin B display a better toxicity profile, and therefore, in spite of their high acquisition cost, may even be cost-effective when compared with conventional amphotericin B.14,15 It must, however, be mentioned that each lipid formulation (liposomal amphotericin B, amphotericin B lipid complex (ABLC), and amphotericin B colloidal dispersion (ABCD)) has markedly distinct biochemical, pharmacokinetic and pharmacodynamic properties. Also, neither ABCD nor ABLC shows a reduced incidence of acute infusion-related side effects compared to the parent compound.16,17

In the 1970s researchers identified several classes of naturally occurring compounds that could inhibit fungal cell wall synthesis, including the echinocandin family of antifungals (echinocandins, cilofungin, aculeacins, pneumocandins, mulundocandins, and WF-11899).18 The echinocandins and pneumocandins, structurally related lipopeptides with a cyclic peptide core and a number of attached lipid chains, proved to be fungicidal in vitro and in animal models. Although the natural product echinocandin B, the first antifungal representative of this class, induced hemolysis, simple substitution of the linoleoyl group with synthetic side-chains resulted in non-lytic analogs. In particular, cilofungin (LY121019), the 4-n-octyloxybenzoyl ECB derivative, appeared very promising; the drug was more than 10-fold less hemolytic than the parent compound, and retained excellent cidal activity against Candida spp. and A. fumigatus. However, cilofungin was abandoned in phase II clinical trials,
because of renal acidosis associated with the polyethylene glycol vehicle in which the drug was given.\(^{19}\)

Several companies have continued their search for semisynthetic analogs with improved pharmacologic properties and identified three water-soluble bioactive derivatives: caspofungin (derived from pneumocandin B\(_\text{9}\), Merck & Co. Inc.), anidulafungin (derived from echinocandin B, Versicor), and micafungin (Fujisawa). These analogs are more potent and display a broader antifungal spectrum than their respective natural counterparts.\(^{20}\) This review aims at summarizing the clinical development and current status of caspofungin, the first in a new class of antifungals, in the treatment of Candida and Aspergillus infections.

**MECHANISM OF ACTION**

All candins exert their action through inhibition of the synthesis of glucan, a critical fungal cell wall component not present in mammalian cells. These agents primarily act as specific and non-competitive inhibitors of \(\beta-(1,3)\)-D-glucan synthase. The resulting depletion of cell wall glucan leads to osmotic shock, ballooning, and ultimately lysis of the cell.\(^{21}\) This is distinct from the azoles and polyenes, which target cell membrane ergosterol (Table 1).

**IN VITRO ACTIVITY**

Caspofungin (Figure 1) displays potent in vitro and in vivo activity against a wide range of yeast and fungi, including most clinically relevant Candida and Aspergillus spp. A large number of in vitro susceptibility studies have shown that caspofungin is equal to or more potent than the azoles itraconazole and fluconazole, the investigational triazoles voriconazole, posaconazole and ravuconazole, amphotericin B, flucytosine and other investigational candins (e.g. LY303366) against Candida albicans, Candida glabrata, and Candida tropicalis.\(^{22-26}\) Caspofungin MIC\(_{90}\) values against azole-susceptible strains are 1 \(\mu\text{g/mL}\) or less; one study even found that all Candida isolates were inhibited at a concentration of 0.78 \(\mu\text{g/mL}\) or less.\(^{24}\) Given its novel mechanism of action, caspofungin could be expected to retain activity against Candida strains with acquired or intrinsic resistance to the currently available classes of antifungals. MIC values against amphotericin B-, flucytosine- or azole-resistant isolates of Candida albicans, Candida glabrata, Candida tropicalis and Candida lusitaniae are consistently less than 2 \(\mu\text{g/mL}\). The activity against Candida krusei is comparable to that of amphotericin B, itraconazole, and posaconazole. However, caspofungin has less or poor in vitro activity against Candida parapsilosis (MIC\(_{90}\) 0.5–4 \(\mu\text{g/mL}\)) and Candida guillermondii (MIC\(_{90}\) 0.5–8 \(\mu\text{g/dL}\)).\(^{27}\)

Caspofungin shows enhanced in vitro activity (MIC\(_{90}\) 0.12 \(\mu\text{g/mL}\)) against clinical isolates of Aspergillus, including A. fumigatus and A. flavus, compared with itraconazole, amphotericin B, and flucytosine.\(^{26}\) These results have been confirmed with Aspergillus isolates obtained from patients included in phase III clinical trials; for all species, MIC\(_{90}\) geometric means are below 1 \(\mu\text{g/mL}\) at 24 h.

The in vitro activity of caspofungin has been confirmed in several animal models.\(^{28-31}\) Efficacious in vivo activity against Candida spp. (including Candida glabrata and Candida krusei) has been shown in a range of murine models. Even in the most stringent model of disseminated candidiasis, a chronically pancytopenic mouse model, caspofungin prolonged survival compared to amphotericin B, and sterilized the kidneys, in mice with disseminated infection, even when therapy was delayed for 24 h. This underlines the fungicidal activity against Candida spp. Caspofungin showed a dosage-dependent tissue clearance of Candida albicans in liver, spleen, kidney, brain, vitreous and lung in a persistently neutropenic rabbit model.\(^{32}\)

When compared to controls, caspofungin also significantly prolonged the survival of immunosuppressed and neutropenic mice with disseminated/pulmonary aspergillosis. It is more difficult to characterize the cidal nature of the activity against Aspergillus. In the presence of caspofungin, blunting and abnormal branching of the hyphae in actively growing areas of the cell are

---

**Table 1. Mechanisms of action of antifungal agents: implications for efficacy**

<table>
<thead>
<tr>
<th>Agent</th>
<th>Site of action on the fungal cells</th>
<th>Activity</th>
<th>Clinical implications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphotericin B</td>
<td>Membrane</td>
<td>Binds to ergosterol; causes cell death</td>
<td>Potent broad-spectrum fungicidal activity</td>
</tr>
<tr>
<td>Azoles</td>
<td>Membrane</td>
<td>Inhibits CYP 450 enzyme responsible for ergosterol synthesis; damages cytoplasmic membrane</td>
<td>Fungistatic activity of variable potency and spectrum</td>
</tr>
<tr>
<td>Caspofungin</td>
<td>Wall</td>
<td>Inhibits glucan synthesis; disrupts cell wall structure and ultimately causes lysis of cell</td>
<td>Potent broad-spectrum antifungal activity; potential for additive effects in combination therapy</td>
</tr>
</tbody>
</table>
observed, primarily at the tips and branching points. These observations are consistent with the mechanism of action, but do not fit the classical definitions of fungicidal or fungistatic. However, data from human and animal models show that the drug is efficacious, even in animals with prolonged immunosuppression. Contrary to the results of Candida animal studies, reduction of tissue colony-forming units (CFUs) correlates poorly with fungal burden in Aspergillus models. Using a quantitative PCR-based assay (qPCR) to monitor disease progression and measure drug efficacy, Bowman et al demonstrated that both caspofungin and amphotericin B reduced the A. fumigatus burden in infected kidney to the limit of detection for the qPCR assay.

Caspofungin was also extremely potent in the prevention and therapy of pulmonary pneumocystosis in immunocompromised animal models, and prolonged survival and reduced tissue burden in murine histoplasmosis. Caspofungin does not have clinically useful activity against Cryptococcus neoformans.

In most studies, susceptibility testing was performed according to a modification of both the National Committee for Clinical Laboratory Standards (NCCLS) method M38-P (for Aspergillus spp.) and method M27-A (for Candida spp.). It is important to remember that in vitro susceptibility testing for echinocandins has not yet been standardized. Therefore, translation of in vitro testing results into in vivo activity remains troublesome. For instance, in the randomized invasive candidiasis study, caspofungin MICs did not predict microbiological outcome; the drug proved to be highly effective against Candida spp. with reduced in vitro susceptibility, including Candida parapsilosis. More recently, alternative methods based on colorimetric assays have been suggested.

To date, there has been no antagonism noted in preclinical studies between caspofungin and other antifungal therapies. Studies evaluating synergy and additivity with caspofungin are being explored in vitro and in animal models. As demonstrated by Bartizal et al, exposing Candida albicans isolates to subinhibitory concentrations of caspofungin did not induce resistance, even after 40 passages. Echinocandin resistance outside the laboratory has not yet been reported.

HUMAN PHARMACOKINETICS

Caspofungin is a very large molecule developed for parenteral use only. In animal studies, oral bioavailability appeared to be extremely poor, less than 0.2% of the drug was absorbed following an oral dose of 50 mg/kg. Metabolism and excretion are very slow processes. Caspofungin is extensively bound to albumin (>95%), and plasma pharmacokinetics are primarily controlled by the rate of distribution from plasma into tissues. Tissue-to-plasma ratios for AUC24 following intraperitoneal administration of [3H]MK-0991 (1 mg/kg) in mice were 16, 3 and 2 for liver, kidney and large intestine, respectively, and 3.5, 0.2 and 0.06 for heart, thigh and brain, respectively. The exposure of the small intestine, lung and spleen was similar to that of plasma. Since the elimination occurs in the absence of distribution equilibrium, the true volume of distribution cannot be estimated. Caspofungin displays non-linear pharmacokinetics, with moderate accumulation with multiple dosing. Average concentrations at steady state with a 50-mg daily regimen are above the target concentration of 1 µg/mL for the entire 24-h dosing interval (determined from in vitro susceptibility testing of clinically relevant fungal isolates) by day 2; a loading dose of 70 mg on day 1 to reach steady state faster is recommended for the treatment of Aspergillus infections. Higher doses of caspofungin, given intravenously as single 150 mg and 210 mg doses or as multiple daily 100-mg doses, have been evaluated in adult volunteers, and proved to be well tolerated. Following multiple daily doses of 100 mg, the AUC24 was approximately 2.5 times that with the 50-mg daily regimen. Unfortunately, information on tissue concentration in humans and mode of action in human tissue is not yet available.

Caspofungin undergoes spontaneous chemical degradation to an open-ring compound, followed by further peptide hydrolysis and N-acetylation. Plasma elimination is slow, with a clearance of 10-12 mL/min. Following single 1-h infusions in healthy men, plasma concentrations decline in a polyphasic manner: a short alpha-phase occurs immediately post-infusion, followed by a beta-phase with a half-life of 9-11 h, and an additional gamma-phase with a half-life of 45 h. This profile allows once-daily dosing. Four weeks after the intravenous administration of radiolabeled caspofungin, approximately 75% of the dose was recovered, equally divided between urine (41%) and feces (34%). Excretion is slow, and the terminal half-life of radioactivity was 12-15 days. Approximately 1.4% of the dose is excreted as parent drug in urine, suggesting that the primary route of excretion is hepatic.
In patients with mild (Child-Pugh score 5–6) and moderate (Child-Pugh score 7–9) hepatic impairment, the AUC is increased by about 20% and 75%, respectively. To date, there are no clinical data on severe hepatic insufficiency (score >9). A reduction of the maintenance dose to 35 mg daily in moderate hepatic disease provides an AUC similar to that obtained in subjects with normal hepatic function receiving the standard regimen.

There are no clinically significant alterations of pharmacokinetics in patients with mild, moderate, advanced or end-stage renal insufficiency. Therefore, no dose adjustment is necessary for patients with any degree of renal impairment. Caspofungin is not removed from the blood by hemodialysis or ultrafiltration; supplemental dosing is not required following hemodialysis.

There are no clinically meaningful alterations in pharmacokinetics with age, gender, or race. Caspofungin has not yet been sufficiently studied in pediatric patients; its use in patients under 18 years of age is therefore not recommended. There are no well-controlled studies in pregnant women, and it is not known whether caspofungin is excreted in human milk.

**DRUG INTERACTIONS**

In vitro studies have shown that caspofungin is neither an inhibitor of nor a substrate for an enzyme in the cytochrome P450 system. Co-administration of caspofungin and itraconazole did not alter the pharmacokinetics of either drug, suggesting that caspofungin is not subject to drug interactions based on CYP3A4 inhibition.

Caspofungin also did not influence the pharmacokinetics of amphotericin B and mycophenolate mofetil.

In clinical studies performed in healthy volunteers, cyclosporin A (CyA) increased the AUC of caspofungin by ~35%, probably due to decreased hepatic uptake, whereas the plasma levels of CyA remained unchanged. In 5 of 12 subjects, co-administration of both drugs resulted in an increase in liver enzymes (alanine aminotransferase, ALT) of less than or equal to threefold the upper limit of normal, which resolved with discontinuation of the product. Pending additional clinical data, co-administration of caspofungin and CyA is currently not recommended by the manufacturer. However, so far, no liver test abnormalities have been reported in patients who have been treated anecdotally with both drugs.

Caspofungin decreased the AUC of tacrolimus by ~25%. However, this problem is manageable through standard monitoring of tacrolimus blood levels and appropriate dosage adjustments.

**CLINICAL DATA**

The efficacy of caspofungin in locally invasive Candida infections is derived from three comparative clinical trials. Two randomized phase II studies (protocol 003 and 004) provide supportive evidence that caspofungin is effective at daily doses of 35, 50 and 70 mg for the treatment of mucosal Candida infections. Protocol 003, a multicenter, randomized, double-blind study performed in Latin America, compared two doses of caspofungin (50 mg and 70 mg) relative to a standard dose of conventional amphotericin B (0.5 mg/kg) for 14 days in 128 predominantly HIV-seropositive patients with endoscopically documented symptomatic Candida esophagitis. Candida albicans was the predominant pathogen (84%), with MICs ranging from 0.06 to 2 μg/mL. All treatment regimens were highly effective in achieving favorable combined clinical and endoscopic responses (modified intention-to-treat analysis), although the response rate (resolution of symptoms and significant reduction in endoscopic lesions 14 days after completing therapy) was highest for those receiving caspofungin at 70 mg (89%) and lowest for the amphotericin B group (63%). Patients treated with caspofungin also had a slightly higher rate of microbiological eradication. In protocol 004, patients with oropharyngeal candidiasis (OPC) or esophageal candidiasis (EC) received caspofungin 35, 50 or 70 mg daily, or amphotericin B 0.5 mg/kg per day, for a minimum duration of 7 days (OPC) to 10 days (EC).

The response rates for each of the three tested doses of caspofungin (complete resolution of symptoms and significant reduction in endoscopic or oropharyngeal lesions) was numerically higher than seen with amphotericin B at 0.5 mg/kg, although the daily 70-mg dose of caspofungin failed to show any additional benefit. Since both studies were designed as proof of concept trials, these protocols could not demonstrate therapeutic equivalence or superiority to amphotericin B.

The information from phase II studies and the pharmacokinetic studies, as well as data from in vitro susceptibility testing, led to the selection of a 50-mg dose for a phase III comparative study of caspofungin versus fluconazole in Candida esophagitis. This study showed that caspofungin 50 mg daily was as effective as fluconazole 200 mg daily. The favorable overall combined response was approximately 80–85% in both treatment arms.

Data on the efficacy of caspofungin in invasive candidiasis (IC) and Candida fungemia have recently been presented. Patients with IC were randomized to caspofungin (70 mg × 1, then 50 mg/day) or amphotericin B (0.6–1.0 mg/kg/day). In the primary analysis, caspofungin was equivalent to amphotericin B in terms of efficacy, although it was far better tolerated than amphotericin B. In a predefined secondary analysis (at least 5 days of therapy), caspofungin proved to be superior to amphotericin B.

The efficacy of caspofungin in aspergillosis comes from the preliminary analysis of an ongoing multicenter, non-comparative phase II salvage study involving 56 immunocompromised patients with a diagnosis of definite or probable pulmonary aspergillosis or definite candidiasis.
Forty-six patients were refractory to at least 7 days of standard anti-Aspergillus therapy, including conventional amphotericin B or a lipid formulation, itraconazole, or an investigationalazole with anti-Aspergillus activity. Half of the patients had received more than 14 days of therapy, and one-third received more than 3 weeks of previous therapy. Ten patients were enrolled because of intolerance to standard antifungal therapy, mainly nephrotoxicity. The site of infection and the underlying conditions are shown in Figures 2 and 3. All patients received an intravenous loading dose of caspofungin of 70 mg, followed by a daily dose of 50 mg for up to 162 days (mean 31 days). An independent expert panel assessed response. A complete (resolution of all attributable symptoms, signs, and radiographic or bronchoscopic abnormalities) or partial (clinically meaningful improvement in attributable symptoms, signs, and radiographic or bronchoscopic abnormalities) response occurred in 41% of the patients who received at least one dose of caspofungin. As expected, patients who did not tolerate previous antifungal therapy had a better outcome (70%) than those who were refractory at enrollment (34%). Favorable outcomes have been documented across all degrees of immunodeficiency, including the persistently neutropenic patient. Compared to a well-balanced historical control group, caspofungin was more commonly associated with favorable outcomes than standard therapy (OR 3.6); this effect was consistent across subgroups in both adjusted and unadjusted analyses. Results from these 56 patients formed the basis for regulatory approval of caspofungin as salvage therapy in invasive aspergillosis. The updated report on the first 90 patients confirmed the efficacy (favorable response rate of 45%).

An international phase III study comparing caspofungin with ambisome in the empirical treatment of febrile neutropenia refractory to broad-spectrum antibacterials is currently being analyzed.

**TOLERABILITY AND SAFETY**

To date, caspofungin has been generally well tolerated both in healthy subjects from the clinical pharmacology studies and in patients with a wide spectrum of diseases and many concomitant medications included in clinical trials. In clinical studies of patients with invasive aspergillosis, caspofungin was well tolerated for treatment durations up to 162 days, and the favorable safety profile was maintained with extended therapy (>28 days). Drug-related clinical adverse experiences were typically mild and self-limited, and included fever, headache, phlebitis, and rash. Among the patients who received 50 mg or 70 mg of caspofungin in Candida studies, phlebitis was the most commonly reported local injection site adverse reaction. In comparative studies, phlebitis occurred with an incidence of 12% and 22% in patients receiving, respectively, caspofungin 50 mg and amphotericin B 0.5 mg/kg. In controlled phase II Candida infection studies, significantly fewer patients in the study arms receiving caspofungin than in the amphotericin B arm developed fever or chills. The clinical safety profile of caspofungin mimicked that of fluconazole in the phase III Candida esophagitis study. Overall, significantly fewer subjects receiving caspofungin than amphotericin B discontinued therapy because of adverse events (4% versus 24% in protocol 003).

The most frequent drug-related laboratory adverse effects were liver function test abnormalities (~10%), which occurred at rates similar to those with fluconazole,

---

**Figure 2.** Underlying disease in patients with invasive aspergillosis. BMT, bone marrow transplant. PSC, peripheral blood stem cell transplantation.
and decreases in hemoglobin and hematocrit. Most of the elevations in hepatic enzyme levels were transient and did not limit therapy. In an in vitro hemolysis assay, caspofungin was found to be relatively non-hemolytic against human red blood cells (similar to distilled water, and less toxic than low doses of amphotericin B). Also, in contrast to the case with amphotericin B, drug-related elevations in serum creatinine were extremely uncommon (1.4%) in caspofungin-treated subjects, and similar to those seen with fluconazole. In the three completed clinical trials for EC and OPC, there were no serious drug-related adverse experiences and few adverse experiences leading to discontinuation of therapy in the caspofungin recipients.47-49

In the Aspergillus salvage study, 14% of patients had adverse reactions that were judged to be related to caspofungin therapy. The following clinical adverse events occurred with an incidence greater than 2%: fever, nausea, vomiting, and phlebitis. Laboratory abnormalities related to caspofungin that occurred in more than one subject included eosinophilia (n=2), proteinuria (n=3), increased alkaline phosphatase (n=3), and hypokalemia (n=2). Overall, three severe adverse experiences were reported as drug-related: one case of hypercalcemia, and one case of pulmonary infiltrates in a transplant recipient who received multiple antimicrobial agents;51 in addition, there was one case of reversible anaphylaxis in the compassionate use program.

CONCLUSION

Caspofungin, the first in a new class of antifungal agents, is currently approved in the USA, Mexico, Brazil, Argentina, Puerto Rico, Peru, New Zealand, Australia and Europe for the treatment of patients with Aspergillus infections refractory to other antifungal therapies, or who are intolerant to these therapies. Approval for Candida infections is pending. Considering all of the efficacy and safety data, caspofungin offers a potential benefit to many patients who have otherwise limited therapeutic options. The availability of fungal cell wall inhibitors will allow future studies examining combination with other antifungal drugs and/or cytokines.54-57

REFERENCES


Caspofungin in the treatment of candidosis and aspergillosis / Maertens et al


