

Caspofungin

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Caspofungin, the first inhibitor of fungal β -1,3 glucan synthesis to receive approval by the United States Food and Drug Administration, is effective for the treatment of mucosal and invasive candidiasis and invasive aspergillosis. It is also active in vitro and in animal models against a number of other filamentous and dimorphic endemic fungi and in animal models of *Pneumocystis carinii* infection. In vitro studies and some animal studies almost always indicate an absence of antagonism when caspofungin is combined with azole or polyene antifungal agents. Caspofungin has an excellent safety profile. Caspofungin may prove to be useful in empirical therapy for suspected invasive fungal infections. Additional clinical trial data that expand our knowledge of the usefulness of caspofungin for these and other mycoses, including its administration in combination with other antifungal agents, is anticipated. Caspofungin is an important addition to the antifungal pharmacopoeia.

The development of inhibitors of the synthesis of fungal cell wall glucan represents an important advancement in antifungal chemotherapy. Known (1,3)- β -D-glucan synthase inhibitors include the acidic terpenoids, the papulocandins, and the echinocandins, cyclic hexapeptides that are N-linked to a fatty acyl side chain [1–3]. Caspofungin (L-743,872, MK-0991), an echinocandin, is the first of these noncompetitive inhibitors to become available in the United States, where it is marketed as Cancidas (Merck), and in Europe, where it is marketed as Caspofungin MSD (Merck). Caspofungin is a water-soluble amphipathic lipopeptide with a molecular mass of 1213 kDa that is a semisynthetic derivative of pneumocandin B0, a fermentation product of *Glarea lozoyensis*. At the time of this writing, caspofungin has received US Food and Drug Administration (FDA) approval for the “treatment of invasive aspergillosis in patients who are refractory to or intolerant of other therapies (i.e., amphotericin B, lipid formulations of amphotericin B, and/or itraconazole)” (p. 6), as well as for the treatment of oropharyngeal and esophageal candidiasis and invasive candidiasis in adults [4].

MECHANISM OF ACTION: THE FUNGAL CELL WALL

Disruption of the fungal cell wall results in osmotic stress, lysis, and death of the microorganism. The cell wall of yeasts includes polymers of glucose, mannose, and *N*-acetylglucosamine, forming the polysaccharides, glucan, mannan, and chitin, respectively [5]. Glucan is a microfibril composed of 3 helically entwined linear polymers of glucose. Glucan is the major component of the fungal cell wall, accounting for 30%–60% of the cell wall of *Saccharomyces* and of *Candida*. In *Saccharomyces*, this polysaccharide predominantly comprises β -1,3 glucan with β -1,6 linkages at branch points [5–7]. The cell wall composition of filamentous fungi differs in some respects from that of yeasts. These differences include a higher concentration of chitin, and, in *Aspergillus fumigatus*, the presence of a poly-*N*-acetylgalactosamine polymer and a novel linear β -1,3/1,4 glucan [8–11].

Glucan synthase is a UDP-glucosyl-transferase located in the fungal cell membrane. Glucan synthesis occurs on the cytoplasmic side of the membrane, with glucan chains then extruded toward the periplasmic space for incorporation into the cell wall [12]. Glucan synthase is a UDP-glucosyl-transferase; β -1,3 glucan is synthesized from UDP-glucose by a membrane-bound enzymatic system. In *Saccharomyces cerevisiae*, this is composed of a catalytic subunit encoded by 2 genes, *FKS1* and *FKS2*, as well as a GTP-bound protein regulatory subunit encoded by *RHO1* [3, 13–15]. Although the viability of *S. cer-*

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evisiae is maintained in the face of single disruptions of either *FKS1* or *FKS2*, suggesting at least partial redundancy of function, disruption of both is lethal [16]. The protein products of *FKS1* and *FKS2*, Fks1p and Fks2p, are highly homologous but are differentially regulated [15].

Deletions and mutations in signaling pathways affect glucan synthesis and the response of fungi to echinocandins. Mutations in the calcium-signaling pathway are associated with hypersensitivity to echinocandins [17]. Such hypersensitivity is also observed in association with deletions in the cell integrity-signaling pathway. These deletions include ones for protein kinase C, the MAP kinase Slt2p, and other components [18].

In contrast to *Saccharomyces* and *Candida*, the species *Cryptococcus neoformans* contains only a single copy of a homologue of *FKS1* whose expression is essential for cell viability [19]. The *FKS* homologue of *C. neoformans* appears, like *FKS2* of *S. cerevisiae*, to be under the transcriptional control of calcineurin [20]. It is observed that exposure to caspofungin is associated with a reduction in β -1,6 glucan of the cryptococcal cell wall. This has raised the possibility of an action in addition to inhibition of β -1,3 glucan synthesis in *C. neoformans* [21]. Genes homologous to *FKS* and *RHO1* of *S. cerevisiae* have been identified in *A. fumigatus*, and, as with *C. neoformans*, only a single homologue of *FKS* has been detected [7, 22]. Homologues of genes encoding glucan synthase in *S. cerevisiae* have also been identified in some dimorphic fungi. One such gene has been observed in *Paracoccidioides brasiliensis*, and 5 have been observed in *Coccidioides immitis* [23]. Resistance to echinocandins is associated with mutations, obtained during in vitro passage in the presence of the drug, in *FKS1* and *FKS2* genes in *Candida* species and *Saccharomyces* isolates [2]. In addition, experimental overexpression of Sbe2p, a Golgi protein involved in the transport of cell wall components, results in resistance to caspofungin in *S. cerevisiae* by an undefined mechanism [24]. Although it had previously been concluded that caspofungin did not appear to be a substrate for multidrug transporters, recently published information indicates that constitutive overexpression of the ABC transporter, Cdr2p, confers resistance to caspofungin [25, 26].

FUNGAL PATHOGENS

Candida Species

In vitro susceptibility: methodological issues. Susceptibility testing methods for β (1,3)-D-glucan synthesis inhibitors have not been standardized, and data correlating results of susceptibility studies with clinical and microbiologic outcomes are very limited. In one large clinical trial of treatment of invasive candidiasis, high MICs observed in a few cases were not predictive of failure [27]. Nonetheless, susceptibility testing of *Candida* species with caspofungin that uses the NCCLS M27-A

microdilution broth method has yielded reproducible results; however, problems of trailing end points with some non-*albicans* species of *Candida* remain [28, 29]. A proposed susceptibility breakpoint for *Candida* species of ≤ 1.0 $\mu\text{g}/\text{mL}$ is under consideration [30].

Caspofungin is highly protein bound ($\sim 97\%$). In spite of this, neither mouse nor human serum had any effect on the susceptibility of a strain of *C. albicans* to caspofungin [31].

In vitro susceptibility: single agents. The IC_{50} for β (1,3)-D-glucan synthesis using membrane preparations of *C. albicans* is 0.6 nmol/L [32]. Caspofungin is fungicidal against *Candida* species, albeit more slowly than is amphotericin B [31]. Resistance is not readily induced in the laboratory; no significant increase in the MIC of caspofungin was seen after 40 passages of a strain of *C. albicans* in subinhibitory concentrations of the drug [31]. The frequency of resistant mutants is 10^{-8} [33].

The MIC_{90} of caspofungin against 400 bloodstream *Candida* species isolates was 0.25 $\mu\text{g}/\text{mL}$; all azole-resistant isolates were susceptible to caspofungin at a concentration of ≤ 0.5 $\mu\text{g}/\text{mL}$ [34]. The MIC_{90} of caspofungin for 3959 *Candida* isolates from 95 centers worldwide was 1.0 $\mu\text{g}/\text{mL}$, and 99% of 157 isolates with high-level resistance to both fluconazole and itraconazole were inhibited by ≤ 1.0 $\mu\text{g}/\text{mL}$ [35]. As in other studies, little difference in MICs was noted across species, with the possible exceptions of some reports of modestly higher concentrations required to inhibit *Candida parapsilosis* and *Candida guilliermondii* [35–37]. This may be related to the trailing phenomenon. *C. guilliermondii* is reported to be less reliably killed by caspofungin [38].

Caspofungin is active in vitro against both azole-susceptible and -resistant *Candida* species isolates, including *Candida krusei*, regardless of the mechanism of azole resistance [37, 39]. Thus, caspofungin is active in vitro against clinical isolates of *C. albicans* with resistance to azoles as the result of alterations in *ERG11* and/or upregulation of *MDR* and *CDR* genes encoding efflux pumps [24]. *Candida lusitanae*, which is commonly resistant to amphotericin B, is susceptible to caspofungin [31, 36, 39].

Caspofungin is fungicidal against *C. albicans* at concentrations close to the MIC; killing is dose dependent up to a concentration of ≥ 16 times the MIC [31, 40, 41]. Both the MIC_{90} and the minimum fungicidal concentration (MFC_{90}) for 180 isolates of *Candida* species, including 40 isolates of *C. albicans* and 20 isolates each of *Candida tropicalis*, *C. parapsilosis*, *C. lusitanae*, *C. guilliermondii*, *C. krusei*, *Candida pseudotropicalis*, and *Candida glabrata*, were 0.25–1.0 $\mu\text{g}/\text{mL}$ [31]. The MFC against 50 isolates of *C. glabrata* was 2.0 $\mu\text{g}/\text{mL}$ [42]. Fungicidal activity was confirmed in experiments in which a 99% reduction in viability of isolates of *C. albicans* and of *C. tropicalis* was achieved within 6–8 h with concentrations of caspofungin of 0.06–0.25 $\mu\text{g}/\text{mL}$ (0.5–2 times the MIC) [31]. One study,

however, has reported that caspofungin lacked fungicidal activity against 11 (46%) of 24 *C. guilliermondii* isolates [38].

The postantifungal effect of caspofungin against *C. albicans* at concentrations of 0.125–1.0 µg/mL lasted 6–8 h [43]. The duration of this effect appears to depend on the MIC for the organism relative to the concentration of drug used. Strain variability independent of the MIC to drug ratio is reported as well [44]. The postantifungal effect observed when *C. albicans* was exposed to caspofungin at concentrations greater than the MIC was >12 h, and it was 0–2 h at concentrations less than the MIC [45]. Caspofungin is active against strains of *C. albicans* and *C. parapsilosis* growing as biofilms [46–49].

In vitro activity in combination with other agents. Time-kill studies of the combination of fluconazole and caspofungin against isolates of *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. krusei*, and *C. neoformans* demonstrated, in each case, indifference [50]. No evidence of antagonism was noted in a separate study of the combination of caspofungin and amphotericin B against *C. albicans* [31]. Caspofungin was synergistic with both fluconazole and voriconazole against *C. parapsilosis*, but indifference was noted in combination with amphotericin B [42].

These studies used planktonic growth conditions. In contrast, fluconazole impaired the activity of caspofungin against *C. albicans* growing in biofilms [51]. The combination of amphotericin B and caspofungin under these conditions was additive [51].

Experimental therapeutic models. Caspofungin was effective in murine models of disseminated infection with *C. albicans*, *C. tropicalis*, *C. glabrata*, *C. lusitaniae*, *C. parapsilosis*, and *C. krusei* [52]. Caspofungin was effective for the treatment of experimental *Candida* infections in both immune-competent and -deficient mice. This includes infections caused by fluconazole-resistant *C. albicans* [52–56]. The sterilization of kidneys in 70%–100% of mice at various dose levels (and in the absence of circulating neutrophils) is evidence of fungicidal activity of caspofungin against *Candida* species in vivo. The combination of fluconazole and caspofungin was indifferent in a murine model of disseminated *C. albicans* infection [57]. The combination of caspofungin and amphotericin B therapy was not significantly superior to amphotericin B therapy alone in mice experimentally infected with azole-resistant *C. albicans* [58].

Clinical results. Three randomized trials have demonstrated the efficacy of caspofungin in the treatment of mucosal candidiasis [59–61]. One randomized trial has demonstrated its efficacy for the treatment of systemic candidiasis [62].

Arathoon et al. [59] randomized 140 patients with mucosal candidiasis, 63% of whom had esophageal involvement and 98% of whom had AIDS, to receive either amphotericin B (0.5 mg per kg of body weight) or caspofungin in doses of 35, 50, or 70 mg; each drug was provided once per day for 7–14 days. The response rates among the caspofungin recipients with ex-

clusively oropharyngeal infections was 84%–93%; it was 67% among those assigned to receive amphotericin B. Favorable clinical and endoscopic responses in patients with esophageal infection were observed in 67%–90% of caspofungin recipients and in 61% of amphotericin B recipients. These differences did not reach statistical significance, in part because this phase 2b study was not powered to determine superiority. It was demonstrated that drug-related adverse events occurred significantly less frequently among the caspofungin recipients.

Villanueva et al. [60] randomized patients with endoscopically demonstrated *Candida* esophagitis to receive either amphotericin B (0.5 mg per kg of body weight) or caspofungin in 1 of 2 doses (50 or 70 mg), with each drug provided once per day. Endoscopically documented clinical success was observed in 63% of amphotericin B recipients, in 74% of those provided caspofungin at a dosage of 50 mg per day, and in 89% in those provided the dosage of 70 mg per day; this difference did not achieve statistical significance. Discontinuation of assigned therapy occurred for 24% of amphotericin B recipients and for only 4% and 7% of caspofungin recipients, respectively.

In another multicenter trial, 175 patients with *Candida* esophagitis were randomized to receive either caspofungin (50 mg q.d.) or fluconazole (200 mg q.d.), with both agents administered intravenously. Ninety-eight percent of patients were HIV infected. The rates of both resolution of symptoms and endoscopically demonstrated clearance of infection in this blinded trial were, respectively, 81% and 85%, whereas drug-related adverse events were observed in 41% and 32% [61]. Only 1 patient, who was receiving fluconazole, had assigned therapy discontinued because of an adverse event. An analysis of outcomes of patients clinically refractory and/or demonstrating in vitro resistance to fluconazole found that the majority were successfully treated with caspofungin [63].

Studies involving patients with systemic infection include that of Mora-Duarte et al. [62]. This study randomized patients with invasive candidiasis (83% had candidemia and 10% had peritonitis) to receive either caspofungin (70 mg on day 1, then 50 mg q.d.) or conventional amphotericin B (0.6–1.0 mg/kg q.d.) for 14 days. Forty-six percent of infections were caused by *C. albicans*, 19% were caused by *C. parapsilosis*, 16% were caused by *C. tropicalis*, 11% were caused by *C. glabrata*, and 2% each were caused by *C. krusei* and *C. guilliermondii*; 3% were mixed. Success was defined as both symptom resolution and microbiological clearance. By a modified intent-to-treat analysis of the 224 eligible randomized patients who received at least a single dose of their assigned therapy, success was observed in 73% of caspofungin recipients and in 62% of amphotericin B recipients ($P = .09$). Among patients who received ≥ 5 days of therapy, success was achieved in 81% of caspofungin recipients and in 65% of amphotericin B recipients ($P = .03$).

All adverse events occurred significantly more frequently among amphotericin B recipients than among caspofungin recipients; discontinuation of therapy as a result of drug-related adverse events was observed for 23% and 3% of recipients, respectively.

Yeasts Other than *Candida* Species

Cryptococcus neoformans. The IC₅₀ for inhibition of synthesis of $\beta(1,3)$ -D-glucan synthesis using membrane preparations of *C. neoformans* is 2.5 $\mu\text{mol/L}$, a concentration 10³ greater than that required with preparations from *C. albicans* [33]. Also, *C. neoformans* is relatively resistant to caspofungin, having a reported MIC₉₀ of $\geq 16 \mu\text{g/mL}$, and caspofungin was ineffective in a murine model of disseminated cryptococcosis [31, 36, 52].

The reason for the relative inactivity of caspofungin against *C. neoformans* remains uncertain. Suggested explanations include the presence of a target with reduced affinity for the drug, a lesser contribution of β -1–3 linkages to glucan structure, and a reduction of caspofungin activity by melanin, a key virulence factor of *C. neoformans* [21, 64, 65].

In vitro studies have nonetheless found evidence of synergy between caspofungin and amphotericin B. In one study, synergy was detected against all 18 strains of *C. neoformans*, including 4 that were fluconazole resistant [31, 66]. The results with the combination of caspofungin and fluconazole were strain dependent, demonstrating either synergism, additiveness, or indifference. Antagonism was not observed [66]. Calcineurin inhibition by FK506 results in synergy between this immunosuppressive drug and caspofungin against *C. neoformans* [20].

Miscellaneous yeasts. The MIC₅₀ for *Trichosporon beigelii* (obsolete nomenclature) [67] has been reported to be 0.5 $\mu\text{g/mL}$ [36]. Nonetheless, a case of trichosporonosis that occurred in a bone marrow transplant recipient while receiving caspofungin was reported [68]. The MIC₉₀ for *Saccharomyces* species is reported to be 1.0 $\mu\text{g/mL}$ [69].

Aspergillus Species

In vitro susceptibility: methodological issues. Determination of MICs for *Aspergillus* and other filamentous and dimorphic fungi is, perhaps even more so than with yeasts, highly method dependent. Nonetheless, the NCCLS Subcommittee on Antifungal Susceptibility Tests has developed a reproducible reference testing procedure (M38-A) for the antifungal susceptibility testing of molds [70]. However, the use of standard broth dilution MIC methods may not be applicable to glucan synthase inhibitors because of their novel mode of action. In an open-label trial of caspofungin therapy involving patients whose disease failed to respond to or was intolerant of previously administered antifungal agents, both patients infected with *Aspergillus flavus* that had MICs of caspofungin of $>64 \mu\text{g/mL}$ had favorable microbiological responses [33].

By the conventional measure of a defined decrease in colony-forming units over time, caspofungin is inhibitory but not fungicidal against *Aspergillus* species [71]. Such measurements are, however, confounded by the multicellular nature of mycelia and by the tendency of these organisms to grow as mycelial masses in liquid culture. Evidence collected via vital staining indicates that, in fact, fungal cells at the hyphal apex and branch points (the active centers for new cell wall synthesis in *A. fumigatus*) are killed by caspofungin [71]. This targeted activity results in marked morphological abnormalities of the cell wall that include highly branched, thick-walled stubby growth of germings and, macroscopically, compact clumps in broth dilution wells [13, 72]. As a consequence, an alternative measure of susceptibility, the minimal effective concentration (MEC), defined as the lowest concentration able to produce the morphological effect, has been proposed [13, 72].

In direct comparisons, the MEC is lower than the simultaneously observed MIC. This divergence broadens with increasing time of incubation as the result of an increase in the MIC while the MEC remains relatively stable [72]. The reported MEC of caspofungin for *A. fumigatus* was ~ 3 -fold lower and that for *A. flavus* was 10-fold lower than the MICs at 24 h, and this difference increased during the next 48 h of incubation [72]. Whether the MEC or any other method of susceptibility is useful to the clinician will ultimately depend upon correlations with therapeutic outcomes in clinical trials and subsequent clinical experience.

In vitro susceptibility: single agents. The IC₅₀ for $\beta(1,3)$ -D-glucan synthesis using membrane preparations of *A. fumigatus* is 9.6 nmol/L [33]. Despite variability in reported results (presumably accounted for by methodological differences), caspofungin has in vitro activity against all species of *Aspergillus*. In studies that have used methods approximating the new NCCLS recommendations for testing *Aspergillus* species, the MIC₉₀ of caspofungin is in the range of 16 $\mu\text{g/mL}$ to $>64 \mu\text{g/mL}$. Other studies that use less-stringent criteria for determination of the MIC report greater apparent susceptibility [42, 73]. When the MEC, rather than the MIC, is measured, the results appear even more favorable, with $>95\%$ of 414 *Aspergillus* isolates (257 isolates of *A. fumigatus*, 30 of *A. flavus*, 29 of *Aspergillus niger*, 21 of *Aspergillus versicolor*, and 16 of *Aspergillus terreus*) inhibited at $\leq 1 \mu\text{g/mL}$; all *A. terreus* isolates were inhibited by $\leq 0.5 \mu\text{g/mL}$ [74]. Itraconazole-resistant strains are susceptible to caspofungin [72].

The postantifungal effect of caspofungin against *A. fumigatus* in vitro is reported to be <1 h [43]. Despite its high degree of binding to proteins, the in vitro activity of caspofungin against *A. fumigatus* is reported to be enhanced by the presence of 5% human serum [75]. In addition, caspofungin and human peripheral blood monocytes (but not neutrophils) exhibited additive antifungal activity against *A. fumigatus* in vitro [76].

***In vitro* activity in combination with other agents.** The combination of caspofungin and amphotericin B is synergistic or additive against *Aspergillus* species in vitro; antagonism has not been reported [31, 77]. Experiments with 20 clinical isolates of *A. fumigatus* found that synergy with caspofungin was observed with either conventional or lipid-complexed amphotericin B [78].

Similar results have been reported with the combination of caspofungin and voriconazole [79, 80]. An examination of 48 clinical isolates (24 isolates of *A. fumigatus*, 10 of *A. terreus*, 9 of *A. flavus*, and 5 of *A. niger*) found synergy between these 2 agents in 88%, an additive effect in 4%, and indifference in the remainder [79]. Another study found that this combination was additive against 10 clinical isolates of *A. fumigatus* [81]. Although combinations of caspofungin with either itraconazole or posaconazole showed synergy against 20 *A. fumigatus* isolates, no significant interaction between caspofungin and either voriconazole or ravuconazole was detected [82]. Other investigators confirmed synergy between caspofungin and itraconazole against 30 of 31 *Aspergillus* species when MICs were examined [83]. When MECs were analyzed, however, the combination showed indifference with 26 of the isolates. Preexposure of *A. fumigatus* germlings to itraconazole resulted in enhanced inhibitory activity on subsequent exposure to caspofungin, and the results were the same when the sequence was reversed [84]. A nonimmunosuppressive calcineurin inhibitor, L-685,818, enhanced the activity of caspofungin against 8 of 11 isolates of *A. fumigatus* [85].

Experimental therapeutic models. Caspofungin improved survival in experimental models of disseminated aspergillosis in intact as well as neutropenic mice [52, 56]. Caspofungin was effective in a model of pulmonary aspergillosis in neutropenic rabbits [86]. Despite improved survival, however, caspofungin-treated animals had an apparent increase in colony-forming units in tissue as well as an increase in serum galactomannan antigen index. These apparently paradoxical results point out the in vivo counterpart of some of the confounding methodological issues in in vitro susceptibility testing discussed above. Thus, evaluation of colony-forming unit measurements in animal models of aspergillosis may provide misleading results. This may be because individual hyphae are composed of many cells separated by septa, and both a mass of fungal cells and an individual cell may yield a single colony-forming unit when cultured [71, 87]. Use of a quantitative PCR assay has demonstrated that colony-forming unit determinations may markedly underestimate the number of *A. fumigatus* cells, particularly at the upper range of hyphal density in tissue infection [87]. For this reason, as well as its greater dynamic range, the quantitative PCR molecular method may be superior to colony-forming unit measurements [87].

Caspofungin was synergistic with voriconazole in a lethal

model of invasive *A. fumigatus* infection in guinea pigs rendered neutropenic and provided corticosteroids [88]. No antagonism was detected when the combination of caspofungin and itraconazole was administered in a neutropenic guinea pig model of disseminated aspergillosis [89].

Clinical results. Caspofungin received FDA approval for salvage therapy in adults with invasive aspergillosis on the basis of a noncomparative trial involving 56 patients with documented invasive infection either refractory to ≥ 7 days of therapy with an appropriate antifungal agent or intolerant of standard therapy [33]. A recent analysis updated to include the first 90 patients in that trial, 83 of whom were assessable, has been reported elsewhere [90]. Eleven percent of the patients were recipients of solid-organ transplants, and 71% had a hematological malignancy; 42% of the patients in this latter group had undergone hematopoietic stem cell transplantation. Twenty-three percent of the total cohort were neutropenic (absolute neutrophil count, < 500 neutrophils/mm³). Seventy-seven percent had definite or probable pulmonary infection; the infection was disseminated in 16%. Most infections were due to *A. fumigatus*, but *A. flavus*, *A. niger*, and *A. terreus* were also represented. The overall efficacy rate was 45%, but it was 56% in patients who received > 7 days of caspofungin therapy. Although 42% of all patients with hematological malignancy had a favorable response, only 14% of those who had received an allogeneic stem cell transplant did so. Failure of resolution of neutropenia was a strong predictor of treatment failure. Therapy was efficacious in the one patient with proven CNS infection. Analysis of 296 historical controls matched to the first 63 patients enrolled found a favorable outcome in only 17% [33].

Caspofungin has been administered in combination with other antifungal agents. Six patients with leukemia whose illness failed to respond to amphotericin B therapy were provided caspofungin together with voriconazole; infection improved in all 6, and there were no drug-related adverse events [91]. Nine patients with hematologic malignancy whose infection had failed to respond to amphotericin B monotherapy had caspofungin added to their regimen, and 1 received triple therapy with voriconazole added. Overall, 5 improved and 1 stabilized [92]. Fifty patients with hematologic malignancies and invasive aspergillosis were treated with caspofungin and liposomal amphotericin B in combination; the infections of 28 failed to respond to ≥ 6 days of therapy with liposomal amphotericin B alone. The response rate was 21% in those with documented and 77% in those with possible invasive aspergillosis. Among patients with documented infection, the response rate was 41% in those provided the combination as primary therapy (vs. 16% in previous cohorts of patients) and only 6% in those provided the combination as salvage therapy. There was no significant toxicity ascribed to the combination [93]. The combination of caspofungin with either conventional or liposomal amphotericin B

icin B was administered to 20 patients with possible and 10 with probable or proven invasive aspergillosis for whom amphotericin B monotherapy had failed [94]. The combination was well tolerated, and a favorable response was observed in 18 patients (60%), including 15 of 20 patients who received intensive chemotherapy for acute leukemia.

Filamentous fungi other than *Aspergillus* species. The in vitro activity of caspofungin against filamentous fungi other than *Aspergillus* species varies more, although the lack of standardization and the use of multiple conditions for susceptibility testing make many of the results difficult to interpret.

Fusarium species are reported to be resistant, but caspofungin and amphotericin B were additive in vitro using the checkerboard method against approximately one-half of isolates tested; in the remainder, synergy was noted; antagonism was not observed [1, 77, 95]. *Rhizopus* species are also resistant, as are dermatophytes [36, 69, 95, 96].

Some reports suggest that caspofungin is active in vitro against some uncommon fungi. For example, ranges of MICs, read at ~50% inhibition of growth, of 6 *Bipolaris* isolates and 5 *Cladophialophora bantiana* isolates were, respectively, 1–2 µg/mL and 2–8 µg/mL [36]. Six isolates of *Pseudallescheria boydii* were inhibited by caspofungin at concentrations of 0.5–4 µg/mL. Two isolates of *Scedosporium prolificans* were tested; their MICs were 4 µg/mL and 8 µg/mL [36]. The MIC for a single isolate of *Acremonium strictum* was 0.5 µg/mL, and the MFC was >16 µg/mL [36]. The range of MICs for 5 *Phialophora* isolates was 1–16 µg/mL, with a geometric mean MIC of 2.8 µg/mL [36]. More than 97% of 37 *Penicillium* isolates were inhibited by ≤1 µg/mL [74].

Dimorphic (Endemic) and Unclassified Fungi

Caspofungin has in vitro activity, as determined by MIC measurements at ~50% growth inhibition, against *Blastomyces dermatitidis*, *Histoplasma capsulatum*, and *Sporothrix schenckii*, but MICs are higher when more conventional end points are used [36, 97]. Melanization of *H. capsulatum* reduces the activity of caspofungin against the yeast form of this organism [65]. Caspofungin was highly effective in an immunocompetent (BALB/c nu/+) model of histoplasmosis, but only modestly effective in models that use athymic mice and pulmonary challenge models in immunologically intact mice [98, 99].

Caspofungin was active in a murine model of coccidioidomycosis, with the MEC possibly a better predictor of therapeutic outcome than the MIC [100]. In this study, mice infected with 1 of 2 strains of *C. immitis*, each with an MEC of 0.125 µg/mL but one with an MIC of 8 µg/mL and the other with an MIC of 64 µg/L, responded equally well to treatment with caspofungin.

The possibility of antagonism between caspofungin and amphotericin B, fluconazole, or itraconazole in a small proportion

of experiments with the endemic fungi was suggested, although synergy was the pattern most often observed [97]. In its transformation from the mycelial to the pathogenic yeast form, *Paracoccidioides brasiliensis* changes its sugar linkages within the glucan polymers from β to α; the organism is nonetheless inhibited in vitro by caspofungin, although at a relatively high concentration [97, 101].

Caspofungin is active in vitro and in the treatment of experimental *Pneumocystis carinii* infection of mice [102]. Although some investigators have concluded that echinocandins are only active against the cyst form of the organism, other evidence indicates activity against the trophozoite form as well [103].

PHARMACOLOGY

Caspofungin has very limited oral bioavailability and is only available for intravenous administration (table 1) [33, 105]. The mean peak and trough serum concentrations after administration of a single 70-mg dose to adults were 12.1 and 1.3 µg/mL, respectively. Daily administration of 50 mg after an initial 70-mg loading dose maintains mean trough concentrations of >1.0 µg/mL, a target concentration chosen on the basis of preclinical studies as likely to be effective in treatment of *Candida* and *Aspergillus* infections [105]. Daily intravenous administration of 100 mg leads to drug accumulation, with a steady state reached in the third week, and achieves an exposure ~2.5 times that observed with a 50-mg daily dose [105, 106]. Preliminary data indicate that a daily dose of 50 mg/m² for children may provide exposure similar to that seen in adults receiving 50 mg per day [107].

An initial α phase, lasting 1–2 h, is associated with distribution of the drug into the extracellular fluid space; the volume of distribution is 9.7 L. The subsequent β phase exhibits log-linear behavior ~6–48 h after infusion and appears to represent a period during which drug is bound to and penetrates tissues, especially hepatic tissues. Despite the continued decrease in serum concentrations with a half-life of 9–11 h, and despite the fact that this phase accounts for most of the plasma clearance of caspofungin, no metabolism or excretion of the drug occurs during this phase. Therefore, plasma clearance is primarily determined by the rate of distribution into tissues. The subsequent γ phase has a half-life of 40–50 h and appears to reflect slow drug release from tissues [33, 105].

Data on tissue penetration of caspofungin in humans are not available, but intraperitoneal administration to mice is associated with liver exposure 16 times that of plasma, whereas kidney exposure is twice that of plasma [108]. Brain penetration was very limited, and the large molecular mass, water solubility, and high protein binding would suggest that penetration into CSF in the absence of inflammation is likely to be limited [33,

Table 1. Caspofungin dosing and pharmacokinetics.

Variable	Dosage or parameter
Patient group, recommended dosage for adults	
Usual	70 mg LD, then 50 mg q.d. ^a
Advanced age	70 mg LD, then 50 mg q.d.
Renal insufficiency	70 mg LD, then 50 mg q.d.
Hemodialysis	70 mg LD, then 50 mg q.d.
Hepatic insufficiency	
Mild ^b	70 mg LD, then 50 mg q.d.
Moderate ^c	70 mg LD, then 35 mg q.d.
Severe	No data
Coadministration of "inducers": efavirenz, nevirapine, rifampin, dexamethasone, phenytoin, or carbamazepine	70 mg LD, then 70 mg q.d.
Pharmacokinetic parameter ^d	
Peak serum concentration, $\mu\text{g/mL}$	12.1
Trough serum concentration, $\mu\text{g/mL}$	1.3
Volume of distribution, L	9.7
AUC (0–24 h), $\mu\text{g} \times \text{h/mL}$	93.5
Half-life, h	
α Phase	1–2
β Phase	9–11
γ Phase	40–50
Protein binding, %	96.5

NOTE. AUC, area under the concentration-time curve; LD, loading dose. Modified from [104] and reprinted with permission from Infectious Disease Alert, American Health Consultants (Atlanta, GA) and [33].

^a On the basis of limited data, a dosage of 70 mg per day also appears to be safe and may be considered in appropriate circumstances. A 100-mg daily regimen is under investigation.

^b Child-Pugh score, 5–6.

^c Child-Pugh score, 7–9.

^d Single-dose studies.

108]. Nonetheless, some patients with CNS aspergillosis have responded to caspofungin therapy [33, 90].

In healthy adults who intravenously received a single 70-mg dose of tritiated caspofungin, 41% of the dosed radioactivity was recovered in urine and 35% was recovered in feces over 27 days [109]. Although overall clearance is ~ 12 mL/min (0.15 mL/kg/min), renal clearance is only 0.15 mL/min. Biliary and renal excretion of unchanged drug are minimal. Caspofungin undergoes spontaneous peptide hydrolysis and *N*-acetylation, and its major degradation product, a ring-opened peptide, lacks antifungal activity. Further metabolism of the hexapeptide structure is believed to involve hydrolysis into constitutive amino acids

Although clearance is modestly reduced in women and in elderly persons, dosage adjustments are not necessary [110, 111]. Similarly, no dose modifications are required in the presence of renal insufficiency, and the drug is not removed during hemodialysis [33]. No change in dosing is indicated in the presence of mild hepatic disease. However, as a consequence

of a 1.76-fold increase in the area under the concentration-time curve (AUC) in the presence of moderate hepatic insufficiency (Child-Pugh score, 7–9), dose alteration is recommended in this circumstance—after the initial 70-mg loading dose, the subsequent daily dose should be 35 mg [112]. Caspofungin is $\sim 97\%$ bound to plasma proteins.

Preclinical studies indicated that caspofungin is neither a significant substrate nor a potent inhibitor of P-glycoprotein or of hepatic cytochrome enzymes. Analysis of pharmacokinetic data from therapeutic trials, however, has found potentially significant reductions ($\sim 20\%$) in the caspofungin AUC with coadministration of efavirenz, nevirapine, rifampin, dexamethasone, phenytoin, or carbamazepine, possibly as the result of induction of either a minor oxidative pathway or of a transport mechanism. As a consequence, the daily dose of caspofungin should be maintained at 70 mg in patients concomitantly receiving one of these agents [33].

Although cyclosporin exposure is not altered by coadministration with caspofungin, the 24-h AUC of caspofungin is

increased by ~35%, possibly as the result of inhibition of uptake into hepatic tissue [33]. Coadministration of caspofungin with tacrolimus results in no effect on caspofungin exposure, but whole-blood tacrolimus levels are reduced by ~20%. It has been suggested that this may result from decreased RBC binding by tacrolimus [113]. There appears to be no pharmacokinetic interaction between caspofungin and mycophenolic acid or its pharmacologically active glucuronide. Caspofungin does not have significant pharmacokinetic interactions with amphotericin B, itraconazole, or 2-hydroxy-itraconazole [33, 111].

SAFETY

Safety data presented to the FDA were primarily based on observations of 274 subjects enrolled in clinical pharmacology studies who received caspofungin for 1–21 days and of 338 patients in therapeutic trials who received the drug for 1–162 days [33, 114]. Of the former cohort, 126 received ≥ 50 mg daily for >7 days, whereas, of the 338 patients enrolled in therapeutic trials, 217 received a similar dose for >7 days. These data indicate that the overall incidence of clinical and laboratory adverse events attributable to caspofungin was similar to that for fluconazole and significantly less than that for amphotericin B. In addition, single doses of 210 mg and daily doses of 100 mg for 2 weeks were well tolerated [106].

Clinical drug-related adverse events occurred in 8 patients (12%). Only 1 (1%) of 109 patients with invasive candidiasis given caspofungin developed a moderate-to-severe, infusion-

related adverse event, and none developed significant nephrotoxicity or hepatotoxicity [62]. In that study, only 3 caspofungin recipients (3%) discontinued therapy because of a drug-related adverse event. The most frequently seen adverse events were fever, flushing, nausea, headache, vomiting, and infusion-related phlebitis, each occurring in <3% of patients. Caspofungin is a basic polypeptide, a class of drugs that may cause histamine release from mast cells when administered intravenously; symptoms consistent with histamine release were seen in 5 (2%) of 274 patients. Allergic reactions have been infrequent, and anaphylaxis is rare.

Eosinophilia occurred in 3% of caspofungin recipients. The incidence of transaminase elevations (all <5-fold the upper limit of normal) was 14% in caspofungin recipients in studies comparing this drug with fluconazole, a frequency that was similar to that seen in the triazole recipients. There was no significant drug-related nephrotoxicity.

Five of 12 healthy subjects who received cyclosporin together with caspofungin had transient elevations of serum alanine aminotransferase levels that did not exceed 3 times the upper limit of normal [33]. No elevation in the serum transaminase levels occurred, however, in 6 patients with invasive fungal infection to whom caspofungin and cyclosporin were coadministered for 2–56 days [115]. Nonetheless, it is recommended that these 2 drugs not be coadministered in the absence of further data, unless the potential benefit is estimated to outweigh the potential risk.

Caspofungin is classified as pregnancy category C; it is not

Table 2. Wholesale costs of systemic antifungal agents used in the treatment of *Aspergillus* infections.

Antifungal drug	Dosage	Cost, US\$ ^a
Amphotericin B deoxycholate	1–1.5 mg/kg q.d. iv	37.18 ^b
Liposomal amphotericin B	3–5 mg/kg q.d. iv	1318.80
Amphotericin B lipid complex	5 mg/kg q.d. iv	824.66
Amphotericin B colloidal dispersion	3–4 mg/kg q.d. iv	480.00
Itraconazole	200 mg iv b.i.d. (given for 3 days),	369.74
	then 200 mg iv q.d.	184.87
	or 200 mg po t.i.d. (given for 3 days)	48.98
Voriconazole	then followed by 200 mg po b.i.d. ^c	32.65
	6 mg/kg iv q12h for 1 day,	340.00
	then 4 mg/kg q12h,	255.00
Caspofungin	followed by 200 mg po q12h (body weight ≥ 40 kg)	50.00
	100 mg po q12h (body weight <40 kg)	25.00
Caspofungin	70 mg iv for 1 day,	463.75
	then 50 mg iv q.d.	360.00

NOTE. Modified from [118]. Reprinted with permission of the publisher.

^a For 1 day's treatment of a 70-kg patient at the highest usual dosage, according to average wholesale price listing in *Drugs Topics Red Book Update*, July 2002 [119].

^b Price of Fungizone (Bristol Myers-Squibb) in *Drug Topics Red Book Update*, July 2002 [119].

^c Oral formulation of the drug is the capsule form.

genotoxic or mutagenic, but is embryotoxic in rats and rabbits [33]. Thus, it should only be used by pregnant women if the potential benefit outweighs the potential fetal risk.

FORMULARY CONSIDERATIONS

Decisions regarding addition of a novel agent to a formulary must take into consideration differences compared with competing agents with regard to safety, efficacy, product formulation, drug interactions, and cost. Other agents used in the treatment of infections due to *Aspergillus* species are itraconazole, voriconazole, and the various preparations of amphotericin B. In the treatment of *Candida* species infections, other agents, including fluconazole and 5-fluorocytosine, must be considered.

Information summarized above indicates that caspofungin is safe, with an incidence of adverse events that is comparable to that of fluconazole and significantly less than that of amphotericin B deoxycholate. It should be noted that safety considerations related to the cyclodextrin carriers limit the intravenous formulation of itraconazole to administration for no longer than 14 days and that the comparable formulation of voriconazole not be administered to patients with a creatinine clearance of <50 mL/min [116, 117]. Caspofungin is efficacious as salvage therapy in patients with invasive aspergillosis, but no comparative clinical trial data are available. Caspofungin compares favorably with fluconazole and with conventional amphotericin B for the treatment of esophageal candidiasis and is possibly superior to conventional amphotericin B for the treatment of invasive candidiasis. In contrast to fluconazole, caspofungin appears to be active against all species of *Candida*.

In contrast to the triazoles, caspofungin is not available as an oral formulation. Drug-drug pharmacokinetic interactions are minimal, a point of differentiation from voriconazole and itraconazole. The wholesale costs of relevant antifungal agents are presented in table 2. The actual costs to the pharmacy budget are, of course, often negotiable.

SUMMARY AND CONCLUSIONS

At the time of writing, caspofungin had received FDA approval as therapy for infections due to invasive aspergillosis in patients whose infections failed to respond to therapy or who were unable to tolerate other agents and for patients with oropharyngeal or esophageal candidiasis. There are no published randomized trials comparing caspofungin with other agents for the treatment of invasive aspergillosis, and, therefore, it is not possible to comment on relative clinical efficacy. Nonetheless, the apparent relative lack of toxicity and of pharmacokinetic drug interactions make caspofungin an attractive agent. Its activity against all *Candida* species and the absence of drug-drug

interactions and nephrotoxicity will make it a useful choice for the initial empirical treatment of patients with suspected or proven invasive *Candida* infections. It may also prove to be useful for the prophylaxis of fungal infections in neutropenic patients, as well as for empirical therapy for neutropenic patients who have persistent fever despite receipt of antibacterial therapy. The rarity of evidence of in vitro antagonism when combined with other antifungal agents makes it a promising candidate for combination therapy, a strategy already used by some clinicians in the treatment of invasive aspergillosis [91–93].

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