New drug classes

Echinocandin antifungal drugs

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The echinocandins are large lipopeptide molecules that are inhibitors of β-(1,3)-glucan synthesis, an action that damages fungal cell walls. In vitro and in vivo, the echinocandins are rapidly fungicidal against most *Candida* spp and fungistatic against *Aspergillus* spp. They are not active at clinically relevant concentrations against Zygomycetes, *Cryptococcus neoformans*, or *Fusarium* spp. No drug target is present in mammalian cells. The first of the class to be licensed was caspofungin, for refractory invasive aspergillosis (about 40% response rate) and the second was micafungin. Adverse events are generally mild, including (for caspofungin) local phlebitis, fever, abnormal liver function tests, and mild haemolysis. Poor absorption after oral administration limits use to the intravenous route. Dosing is once daily and drug interactions are few. The echinocandins are widely distributed in the body, and are metabolised by the liver. Results of studies of caspofungin in candidaemia and invasive candidiasis suggest equivalent efficacy to amphotericin B, with substantially fewer toxic effects. Absence of antagonism in combination with other antifungal drugs suggests that combination antifungal therapy could become a general feature of the echinocandins, particularly for invasive aspergillosis.

Fungi are flourishing in man. Up to 7% of patients dying in European teaching hospitals have invasive aspergillosis, and *Candida* spp are frequent causes of nosocomial infection.[1,2] Specific patient-groups have very high frequencies of fungal infection: 15% of allogeneic haemopoietic stem-cell transplant recipients have an infection;[3] about 20% of lung-transplant recipients are colonised and infected;[4] about 60% and 20% of AIDS patients in the developed world have *Pneumocystis carinii* pneumonia or oesophageal candidiasis, respectively;[5] cryptococcal meningitis is present in about 30% of people with AIDS in Africa and southeast Asia;[6] and *Penicillium marneffei* infections are present in about 30% of people with AIDS in southeast Asia.[7] Many factors account for these substantial increases in infection, including better management of other complications of immunosuppression, novel and more aggressive immunosuppressive regimens, enhanced survival in intensive care, a high frequency of instrumentation and catheterisation, more awareness by clinicians, better diagnostic approaches, and increased use of antibiotics.

To date, only three classes of antifungal drugs have been available for systemic fungal infections: the polyenes (amphotericin B); the azoles (ketoconazole, itraconazole, fluconazole, and voriconazole); and flucytosine. Although many of these drugs have advanced the management of fungal infections, failure rates remain high,[4] and emergence of intrinsically resistant fungi is a growing problem.[8,9] Introduction of the echinocandins is, therefore, very welcome. Here, the background to this new class of antifungal drug is reviewed, as well as the data lending support to their registration and their probable clinical use.

Origins of the echinocandins

The lead compound for anidulafungin (LY303366; figure 1) was identified in 1974.[10] In 1989, the compound that led to caspofungin (MK991) was reported,[11] and the precursor of micafungin (FK463) was identified in 1990.[12] Several other echinocandin-like compounds have been described, some of which are semisynthetic derivatives of the natural fermentation product, including enfumafungin, the arbocandins, the papulacandins, pneumocandin B, arundifungin, and HMR 3270 (chemically derived from deoxymulundocandin; Hodgson J, Aventis Pharma, personal communication).[13–17] A less active echinocandin B analogue that got to clinical trials is cilofungin, but this particular molecule was difficult to prepare and the formulation was toxic.

Glucan synthase protein complex

The target of the echinocandins is the synthetic cell-wall enzyme complex β-1,3-D-glucan synthase.[18] Fungal cell-walls are rigid structures that consist of large polysaccharides β-(1,3)-D-glucan, β-(1,4)-D-glucan, β-(1,6)-D-glucan, chitin, mannan or galactomannan, and α glucans and various glycoproteins.[19,20] Although fungi are eukaryotes like human beings, the cell-wall is not shared by mammalian cells, and therefore represents a good target for antifungal drugs.

Figure 2 is a diagrammatic representation of the glucan synthase protein complex, and its regulatory network. The short name of the gene encoding β-(1,3)-glucan synthase is *FKS1*, and its deletion yielded a yeast phenotype that was hypersensitive to tacrolimus.[21] Many other mutant phenotypes led to cloning of several genes that were all found to be identical to *FKS1*.[18,22] Diversity of phenotypes led to the cloning of a closely related gene *GSC2* (also known as *FKS2*, which has 88% identity to *FKS1* at the aminoacid level).[22] Deletion of both these genes is lethal in

Search strategy and selection criteria

All papers on echinocandins referenced in MEDLINE were accessed and read. Keywords included the names of each drug and their original code numbers, "Candida", "Aspergillus", and "biofilms". All conference abstracts from the Interscience Conference on Antimicrobial Agents and Chemotherapy from 1998 onwards were also accessed. Additional information was sought from public-access documents and press releases.

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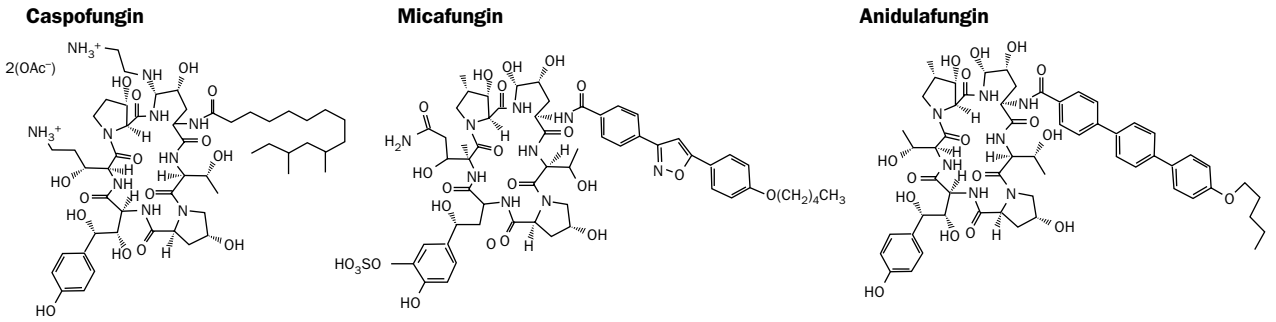

Figure 1: **Chemical structures of caspofungin, micafungin, and anidulafungin**

Saccharomyces cerevisiae, and mutations in *FKS1* can confer caspofungin resistance.[23] Products of *FKS1* and *FKS2* are, at present, thought to be alternate subunits of the β-(1-3)-glucan synthase enzyme complex.[24] Orthologues of *FKS1* or *FKS2* are recorded in all fungi studied to date, with percentage identity varying from 56% (*Cryptococcus neoformans*) to 83% (*Candida glabrata*). A catalytic subunit of β-(1,3)-glucan synthase is present in and expressed during cell-wall formation of the cyst form of *Pneumocystis carinii*.[25] Substantial work has gone into understanding where echinocandins bind to the glucan synthase enzyme complex, but this question is not fully resolved.

Control of β-(1,3)-glucan synthesis has been studied in *S cerevisiae* and pathogenic fungi.[26] *FKS1* transcription is cell-cycle regulated, and linked to cell-wall remodelling. *FKS2* transcription is calcineurin-dependent.[14] A key regulatory protein seems to be the product of *RHO1*, which interacts not only with Fks proteins but also with protein kinase C.[27] This protein is a well studied regulator of the mitogen-activated protein (MAP) kinase cascade and the actin cytoskeleton assembly pathway in yeast. Because of the interaction with multiple proteins, Rho1p is thought to be a key switch, driving or arresting the synthesis of β-(1,3)-glucan. Rho1 seems to be dependent on guanine-nucleotide exchange factors, which are provided by Rom1 and Rom2 proteins. Rom2p is activated by the cell-wall-associated signalling glycoproteins Wsc1p and Mid2p. Activation of Rho1p not only activates β-(1,3) glucan synthase but also results in activation of the MAP kinase cascade and affects actin synthesis. Thus, the pathways and interactions between all regulatory proteins are complex, and they are not yet fully understood with respect to cell-wall assembly. The complexity is emphasised by the demonstration of resistant mutants overexpressing a Golgi protein involved in transport of cell-wall components (Sbe2p).[28]

This regulatory pathway is probably important in understanding the absence of activity of the echinocandins against *Cr neoformans*.[29] The *FKS1* gene in this organism is single copy and essential. Binding of present echinocandins could be poor because of structural differences. However, substantial enhancement of echinocandin activity arises in vitro against *Cr neoformans* when calcineurin inhibitors such as tacrolimus are used in combination.[30] Tacrolimus-binding occurs through a protein known as FKBP12, and when the gene coding for this protein is deleted, *Cr neoformans* becomes sensitive to caspofungin. This finding emphasises the importance of the calcineurin pathway for echinocandin activity, at least for some fungi, as it seems to be for azole activity. Susceptibility and killing of *Candida albicans* is enhanced by combination of fluconazole with ciclosporin.[31]

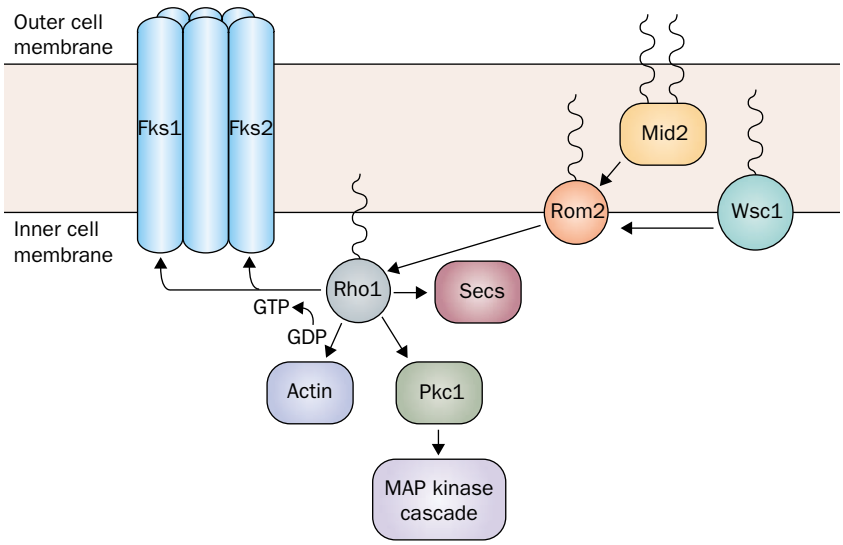

Figure 2: **Diagrammatic representation of the fungal cell membrane**
Proteins forming the β-(1,3)-D-glucan synthase complex (Fks1p and Fks2p) are shown, together with some proteins from the regulatory network.

Chemistry

Echinocandins are large lipopeptide molecules. All molecules in clinical use or development are amphiphilic cyclic hexapeptides with an *N*-linked acyl lipid side-chain and a molecular weight of about 1200.[14,32] Their structures are shown in figure 1. The aminoacid composition of these molecules is unusual, since dihydroxyornithine, 4-hydroxyproline, dihydroxyhomotyrosine, and 3-hydroxy-4-methylproline complement threonine in the peptidic nucleus. Caspofungin has a fatty-acid side-chain, micafungin a complex aromatic side-chain (3,5-diphenyl-substituted isoxazole), and anidulafungin an alkoxytriphenyl (terphenyl) side-chain. Presumably, the side-chain intercalates with the phospholipid bilayer of the cell membrane. Caspofungin (acetate) is freely soluble in water and methanol, and slightly soluble in ethanol.[33] Micafungin (sodium) is freely soluble in water, whereas anidulafungin is not.

Formulations

All echinocandin preparations that have been used to date are for intravenous use only. Caspofungin is licensed for use in the USA and most of Europe, and micafungin in Japan (table 1). Caspofungin is presented as a lyophylised white powder and excipients include sucrose, mannitol,

Drug	Manufacturer	Current status
Caspofungin (MK991, Cancidas)	Merck	Approved
Micafungin (FK463)	Fujisawa	Approved
Anidulafungin (LY303366, VER002)	Vicuron	Phase 3
HMR 3270	Indevus	Phase 1
Cilofungin	Lilly	Discontinued

Table 1: **Echinocandins in development and their status**

acetic acid, and sodium hydroxide.[33] Once reconstituted, this formulation has a pH of 6·6 and is incompatible with dextrose. The drug is generally given by slow intravenous infusion over about 1 h. Caspofungin can be stored (refrigerated) for up to 24 h after reconstitution and dilution before administration. Micafungin is prepared as a powder ready for reconstitution. Excipients include lactose, citric acid, and sodium hydroxide, and once reconstituted the pH of micafungin for infusion is 5–6. Reconstituted micafungin solution is stable at room temperatures for 48 h, if protected from light. Micafungin can be administered with any intravenous infusion. Anidulafungin is provided as a lyophilised powder for reconstitution before infusion.

Pharmacokinetics

The echinocandins have various common features with respect to pharmacokinetics (table 2). At present, all compounds are insufficiently bioavailable for oral use (<0·2% for caspofungin).[33] In a study in volunteers with AIDS given oral anidulafungin in doses up to 500 mg a day, peak plasma concentrations of 753 ng/mL were achieved,[32] but this amount was insufficient to give consistently good results for oropharyngeal candidiasis.

All echinocandins have linear kinetics after intravenous administration. After use, the Cmax of caspofungin (70 mg) and micafungin (75 mg) are similar and are considerably higher than that of anidulafungin (table 2).[32–49] Caspofungin and micafungin have similar beta half-lives, with limited intervolunteer variation. These plasma half-lives are determined mainly by redistribution of drug. The volume of distribution of anidulafungin is greater than for micafungin (and unavailable for caspofungin), as is clearance. Steady-state is achieved for anidulafungin after a loading dose and one subsequent dose; for micafungin it is obtained after 4 days of treatment, but for caspofungin not for more than 2 weeks after initiation of the drug. The steady-state area under the curve of caspofungin and micafungin are similar, whereas that of anidulafungin is much less, at nearly equivalent doses.

One of the distinguishing features of the three echinocandins is their different protein binding (table 2). High protein-binding could limit the amount of drug available for activity. However, only a small amount of micafungin is covalently bound to albumin. Several antifungal drugs are highly protein-bound, including amphotericin B and itraconazole, by contrast with the water-soluble drugs fluconazole and flucytosine. Additionally, the concentrations in cerebrospinal fluid of both amphotericin B and itraconazole are low, but these drugs are effective for fungal meningitis.[50] Therefore, the relevance of drug-protein binding is not yet clear.

Echinocandins are degraded mainly in the liver (also in the adrenals and spleen) by hydrolysis and *N*-acetylation.[33] After the initial distribution phase, hepatic uptake—and therefore degradation—is slow (for caspofungin and micafungin), leading to a long terminal half-life. Extensive uptake by red-blood cells was noted for micafungin. Two uncommon metabolites from micafungin have antifungal activity. These degradation products are excreted slowly over many days, mainly in the bile. Results of radiolabel studies suggest that the liver, renal cortex, and skin contained most residual drug or metabolite.

There is a slight increase in exposure to caspofungin in patients in renal failure, not related to renal excretion or plasma protein-binding.[33] None of the compounds can be dialysed, and so no adjustment is necessary for patients who need renal replacement treatment. Dosage reduction (to 50% daily dose after a standard loading dose) is recommended for patients with severe hepatic dysfunction, only for those receiving caspofungin,[43] although this recommendation is not backed by clinical data.

A study designed to establish the maximum-tolerated dose of anidulafungin in man—with a maximum loading dose of 260 mg followed by 130 mg daily—was unable to establish this maximum dose (no overt toxic effects).[44] Doses as high as 8 mg/kg of micafungin have been administered to a few haematology patients as part of a carefully controlled dose-escalation study without apparent ill-effects, so the maximum tolerated dose of micafungin has not been established.[45,46] No such work has been done in human beings with caspofungin, but in monkeys, evidence of hepatic necrosis associated with raised transaminases was seen at 5 and 8 mg/kg daily, but not at 2 mg/kg day.[33]

Virtually no experience has been accrued with caspofungin and children,[33] although data suggest that a higher dose might be needed compared with adults.[51] Micafungin has been extensively assessed in young children, but not yet premature neonates. The pharmacokinetics of micafungin in children are similar to in adults.[47] Elderly patients metabolise caspofungin slightly more slowly than younger adults, but need no dosage alterations. No ethnic differences in exposure have been reported with caspofungin or micafungin.

	Caspofungin	Micafungin	Anidulafungin
Variable			
Cmax at 70–75 mg/day (μg/mL)*	12·1 (11·1–13·2)	10·9 (SD 1·5)	3·44
AUC_{0-24} (steady state) (μg h^{-1} mL^{-1})	100·5 (87·9–114·8)	111·3 (SD 14·1)	44·4
Beta $t_{1/2}$ (h)	10·6 (SD 1·1)	11–17	18·1
Clearance (mL/min)	10–12·5	~10·5	12·5–19·2
Volume of distribution (L/kg)	†	0·26	0·57
$t_{1/2}$ hepatic impairment (h)	NA (prolonged)	14·4 (SD 0·8)	34–42
$t_{1/2}$ severe renal impairment (h)	NA‡	14·2 (SD 1·5)	33–42
Protein binding	96%	99·8%	84%
Urinary concentration (% of plasma)	1·4%	0·7%	<0·1%
Cerebral spinal fluid concentration (% of plasma)	?low	?low	<0·1%

Data are mean (SD), median (IQR), range, or percentage. Data derived from multiple sources and trials, therefore are not always directly comparable. NA=not available. $t_{1/2}$=half life. *Anidulafungin given as loading dose of 150 mg, followed by 75 mg daily, caspofungin at 70 mg/day. †Data not provided because tissue uptake is complex and under investigation. ‡Probably similar to volunteers.

Table 2: **Key pharmacokinetic variables of the echinocandins in clinical use**

Drug interactions

Since the echinocandins are poor substrates for the cytochrome P450 enzymes, and are not substrates for intestinal or tissue P-glycoprotein, fewer drug interactions are described for these molecules than for the azoles. Slight increases in caspofungin clearance have been seen with powerful inducers or inhibitors of hepatic metabolism, such as efavirenz, phenytoin, nevirapine, nelfinavir, carbamazepine, and dexamethasone, so a slight increase in daily caspofungin dose (70 mg) is appropriate. A bilateral interaction of caspofungin with rifampicin has been recorded, probably as a result of excretion through the biliary system, which results in additional exposure to both compounds (http://www.aspergillus.man.ac.uk). A slightly reduced exposure to tacrolimus (20%) was seen with coadministration of caspofungin,[48] and monitoring of tacrolimus concentrations was recommended. Caspofungin and ciclosporin do seem to interact,[33] resulting in raised caspofungin plasma concentrations (35% increase in area under the curve) but no change in amount of ciclosporin in whole blood. The mechanism of this interaction is unclear, but data in rats suggest that ciclosporin limits uptake of caspofungin into the liver.[33] In volunteers, this interaction resulted in raised liver function tests, but its clinical importance is unclear. No interactions were noted with other antifungal drugs such as itraconazole and amphotericin B,[30] and no interaction was seen between caspofungin and mycophenolate.[33]

No drug interactions have been described with micafungin and other highly protein-bound compounds including warfarin, diazepam, salicylic acid, and methotrexate. Micafungin only substantially amplifies free bilirubin at micafungin concentrations three to 30 times those in plasma. Two volunteer studies with a combination of ciclosporin and micafungin showed no effect on either drug; the same was also seen with tacrolimus.[41]

Results of a combination study of anidulafungin and ciclosporin in healthy volunteers showed a slight increase in exposure to anidulafungin.[39] Two out of 12 participants had raised liver function tests that resolved, and the association between anidulafungin and ciclosporin dosing in these individuals was not clear. The mechanism of this interaction is not known because no effect has been reported of anidulafungin on the metabolism of ciclosporin by human microsomes.[49]

Antifungal spectrum

The echinocandin antifungal spectrum is restricted to *Candida* spp and *Aspergillus* spp, with few exceptions (table 3). All three compounds are fungicidal in vitro and in vivo against most isolates of *Candida* spp, and fungistatic against *Aspergillus* spp. Cross-resistance with polyenes and azoles has not been shown and is not anticipated on the basis of the mechanism of action of the echinocandins. Methods for susceptibility testing of the echinocandins are not yet developed. Alternative media, such as antibiotic medium 3 (Becton Dickinson, Sparks, MD, USA), could be superior for susceptibility testing of *Candida* spp because of improved growth and clearer endpoints. Preliminary studies have shown low inocula ($0{\cdot}5–2{\cdot}5\times10^3$) to be best, with a 100% endpoint. In susceptibility testing of *Aspergillus* spp, some growth inhibition is seen at low drug concentrations; trace growth is recorded at all amounts of drug. Different endpoints have therefore been defined for testing *Aspergillus* spp and echinocandins.

Activity against *Candida* spp

Minimum inhibitory concentrations of all three echinocandins are much lower than for amphotericin B and fluconazole against all common *Candida* spp except *Candida parapsilosis* and *Candida guilliermondii*, for which they are similar. Typical values for *C albicans* are 0·004–0·015 mg/L.[52–61] For *C parapsilosis* and *C guilliermondii*, minimum inhibitory concentrations are typically 0·5–2·0 mg/L.[52–61] Most isolates of *Candida* spp are killed at concentrations similar to those that inhibit growth, but about 10% are tolerant, depending on the species and drug.[56,57] Killing is very rapid, as ascertained by flow cytometry.[59] Furthermore, a postantifungal effect is present.[60,61] Considerable interisolate variation is noted in both minimum inhibitory and fungicidal concentrations to all three echinocandins,[57] which if validated for clinical or in-vivo outcome, could have important implications for selection of the optimum agent for treatment.

In-vivo data broadly accords with in-vitro data. All models have been done with *C albicans* (including fluconazole-resistant isolates),[62–69] with the exceptions of *C glabrata*,[70,71] *Candida krusei* (Henkel T, Vicuron, personal communication),[70] and *Candida tropicalis*.[71] Sterilisation of tissues was the norm in these models, with larger doses and longer durations of treatment needed in chronically neutropenic animals infected with *C albicans* or *C tropicalis*.[63,72] Caspofungin was not effective in reduction of colony counts in a neutropenic *C glabrata* model, and was not very effective in immunocompromised *C krusei* models.[70]

Some work has explored the effect of caspofungin on candida biofilm formation and destruction.[73,74] While fluconazole was completely ineffective and antagonised caspofungin, treatment with caspofungin of established biofilms resulted in morphological alterations, an effect enhanced with amphotericin B co-incubation.

Activity against *Aspergillus* spp

For *Aspergillus* spp, inhibition of growth is detectable at very low concentrations—eg, 0·008 mg/L—of echinocandin drug in some systems, but inocula, media,

Highly active	Very active	Some activity	Inactive
Candida albicans	*Candida parapsilosis*	*Coccidioides immitis*	Zygomycetes
Candida glabrata	*Candida gulliermondii*	*Blastomyces dermatididis*	*Cryptococcus neoformans*
Candida tropicalis	*Aspergillus fumigatus*	*Scedosporium* spp	*Fusarium* spp
Candida krusei	*Aspergillus flavus*	*Paecilomyces variotii*	*Trichosporon* spp
Candida kefyr	*Aspergillus terreus*	*Histoplasma capsulatum*	
*Pneumocystis carinii**	*Candida lusitaniae*		

Highly active implies very low minimum inhibitory concentrations with fungicidal activity and good in-vivo activity, Very active implies low minimum inhibitory concentrations, but without fungicidal activity in most instances. Some activity implies detectable activity, which might have therapeutic potential for man (in some cases in combination with other drugs). Inactive implies no intrinsic activity. There are usually some differences between individual isolates within a species and there might be significant differences between echinocandins. *Only active against cyst form, and probably only useful for prophylaxis.

Table 3: **Range of activity of the echinocandins**

Study design	Fungal disease	Number of patients	Daily doses (mg)	Comparator	Response	Comments
Caspofungin						
Primary, double-blind dose comparison[103]	Oesophageal candidiasis	128	50 and 70	AmB 0·5 mg/kg	50 mg (85%), 70 mg (96%), and AmB (72%)	AmB dose modest, end of treatment combined clinical and endoscopic response rates
Primary, double-blind dose comparison[104]	Oropharyngeal and oesophageal candidiasis	143	35, 50, and 70	AmB 0·5 mg/kg	35 mg (74%), 50 mg (91%), 70 mg (78%) and AmB (63%)	AmB dose modest, endoscopic verification of response
Primary, double-blind[105]	Oesophageal candidiasis	177	50	Fluconazole 200 mg/day	85% *vs* 86%	Higher relapse rate with caspofungin (28% *vs* 17%, p=0·19)
Salvage[111]	Invasive aspergillosis	90	70 (loading) then 50	None	37/83 (45%)	High proportion of previous treatment failures
Double-blind[109]	Invasive candidiasis	224	70 (loading) then 50	AmB 0·6–1·0 mg/kg	83/109 (76%) *vs* 90/115 (78%)	More toxic effects with amphotericin B, similar species responses
Micafungin						
Double-blind[115]	Prophylaxis in HSCT	882	50	Fluconazole 400 mg	80% *vs* 73·5% (p=0·025)	Large study with benefit across all populations and subgroups
Primary, dose comparison[106]	Oesophageal candidiasis	119	12·5–100	None	97·2% endoscopic response rates if >50 mg/day and >10 day therapy	Worse outcome in those with severe disease
Primary, salvage, and combination*	Invasive candidiasis or candidaemia	142	50–100	None	83% candidaemia, 63% invasive candidiasis	Response rate similar for all species, lower for salvage therapy
Primary, open[114]	Various documented fungal infections	70	12·5–150	None	Candidaemia (100%), oespophageal candidiasis (71%), IPA (60%), CNPA (67%), aspergilloma (55%)	The wide range of dose and heterogeneity of the disorders makes assessment difficult
Salvage, combination[112,113]	Invasive aspergillosis	290	50–100	None	37%	Most patients also given AmB in combination, poor outcome population
Anidulafungin						
Primary, dose comparison[107]	Oesophageal candidiasis	36	50 (loading) then 25 and 70 (loading) then 50	None	Endoscopic response rates 81% (50/25) and 85% (70/35)	Clinical response rates slightly higher in the higher dose group
Primary, randomised[108]	Oesophageal candidiasis	~600	NA	NA	NA	Very large study
Primary, open, dose comparison[110]	Candidaemia or invasive candidiasis	120	100 (loading) then 50, 150 (loading) then 75, and 200 (loading) then 100	None	88% (200/100), 89% (150/75), and 81% (100/50) end of treatment response rates	Slightly better response rates at upper doses

AmB=amphotericin B. HSCT=haemopoeitic stem-cell transplantation. NA=not available. IPA=invasive pulmonary aspergillosis; CNPA=chronic necrotising pulmonary aspergillosis *Reusch M, Fujisawa, personal communication.

Table 4: **Pivotal clinical efficacy studies concluded**

addition of sera to the test, and endpoints have differed, making comparisons difficult.[54,55,75–79] Different species could be more or less susceptible to echinocandins—eg, *Aspergillus flavus* seems to be less susceptible to anidulafungin.[76] Results of studies of the effects of caspofungin on *Aspergillus fumigatus* in vitro suggest patchy killing of hyphae, probably of actively growing cells that are remodelling their cell walls.[80,81] Micafungin exposure alters the morphology of the cell wall as seen by electron microscopy; the inner fibrillar layer is lost by 12 h but begins to recover by 24 h, consistent with new cell-wall formation.[82] The outer layer returns to almost normal by 24 h, and there is complete regrowth of hyphae. If subcultured from broth to agar after echinocandin exposure and damage to the cell wall, poor recovery suggests fungicidal activity.[76] However, in animals, all three compounds are effective in improving survival in otherwise lethal models of invasive aspergillosis, but with persistently high counts of *Aspergillus* spp in tissue.[83–90] Substantially higher tissue burdens were seen with *Aspergillus terreus* compared with *A fumigatus*.[84] These findings accord with all three echinocandins being fungistatic against *Aspergillus* spp.

Activity against other fungi

The echinocandins are highly active against *P carinii*.[91–93] Glucan synthase is only expressed in the cyst form of the fungus,[25] and data in animals suggest a good prophylactic effect of the echinocandins at very low doses, but modest treatment effect.[92] It takes 4 days for turnover of cysts in rats,[91] and so in very ill patients any treatment effect would probably be insufficient.

The echinocandins have modest activity against several other organisms (table 3). Caspofungin was effective in extension of survival in experimental infections with *Coccidioides immitis*, and slightly reduced organ burdens in a dose-dependent manner.[94] Despite in-vitro activity,[77] a worse result was seen with experimental histoplasmosis, with only a marginal effect noted.[95] Other fungi that the echinocandins might be active against include *Scedosporium* spp, *Alternaria* spp, *Bipolaris* spp, *Cladophialophora bantiana*, *Phialophora* spp, *Exophiala* spp, *Fonsecaea pedrosoi*, *Paecilomyces variotii*, *Acremonium strictum*, and *Blastomyces dermatididis*.[33,55,77] The echinocandins are not active against *Cr neoformans*, *Trichosporon* spp, *Fusarium* spp, or any Zygomycetes.[33,55,77,78]

Combination studies

Many investigators have looked at combinations of antifungal drugs in the laboratory and in animals with infection.[96–102] For very ill patients, or those with disease that cannot be eradicated, combinations of drugs are attractive, as long as antagonism is not noted. Fortunately, antagonism has not been recorded with any echinocandin-azole or echinocandin-amphotericin B combination in vitro or in vivo. Results of most studies suggest only modest additive effect, with synergy occasionally seen, with both *Candida* and *Aspergillus* spp.

Clinical studies

Clinical development of the echinocandins has explored indications appropriate for an intravenous drug, including oesophageal candidiasis, invasive candidiasis and candidaemia, invasive aspergillosis, and prophylaxis of invasive fungal infection. The first clinical trials that were completed compared amphotericin with three different doses of caspofungin in the treatment of oesophageal candidiasis in patients with AIDS (table 4).[103,104] Caspofungin 35 mg daily is inferior to 50 mg and 70 mg daily, and both these doses were superior to a subtherapeutic dose of amphotericin B (0·5 mg/kg), although confidence intervals were wide.[103,104] Major endoscopic improvement was needed for a response. Caspofungin 50 mg daily was equivalent to fluconazole 200 mg daily.[105] Results of a phase 2 dose-comparison study with micafungin (25–100 mg daily) in 120 patients with oesophageal candidiasis showed the best clinical response rates with doses of 25 mg or more daily, and better endoscopic response rates in those receiving 75 mg or more daily.[106] Overall response rates were similar to those seen with fluconazole.[116] Good results were also seen with two doses of anidulafungin in endoscopically-proven oesophageal candidiasis.[107] A randomised trial of anidulafungin and fluconazole in 600 patients with oesophageal candidiasis has completed enrolment.[108] In those who failed fluconazole because of resistance, good response rates were seen with caspofungin.[117] Results of these studies clearly show the efficacy of the echinocandins in the treatment of a serious mucosal fungal infection.

In a randomised study of primary treatment of invasive candidiasis and candidaemia, treatment with caspofungin was equivalent to amphotericin B.[109] The caspofungin dose used was the standard loading dose of 70 mg followed by 50 mg a day, and this regimen was compared with amphotericin B (0·6–1 mg/kg). Response rates in both arms were 76–78%,[109] which compare favourably with previous data for fluconazole and amphotericin B.[118,119] Caspofungin was less toxic than amphotericin B, and was effective for all species. A large open study of micafungin for the same indication (but mostly in patients intolerant of or failing other treatment) has been concluded (Reusch M, Fujisawa, personal communication). To date, 173 patients were enrolled, 119 with candidaemia and 54 with invasive candidiasis. Some received primary treatment with micafungin, others salvage therapy, and others a combination with other drugs, usually lipid-associated amphotericin B. All outcomes were determined by an external expert. The overall response rate was 83% in patients with candidaemia and 63% in those with invasive candidiasis (Reusch M, Fujisawa, personal communication). A phase 2 dose-comparison study of anidulafungin has completed enrolment of 120 patients with response rates of 81–89%, with very few adverse events.[110]

In these studies, no discernible differences were noted in response rates between different species of candida that cause disease, which suggests that the higher minimum inhibitory concentrations of the echinocandins to *C parapsilosis* might not be clinically important. These data need confirmation in patients with more challenging candida infection—eg, endocarditis or persistent infection with neutropenia. Details of response in so-called sanctuary sites such as the eye, vegetations, urine, mediastinum, and meninges have not yet become available. The broad candida spectrum, rapid killing, and good clinical results imply that the echinocandins will become the treatment of choice for invasive candidiasis and candidaemia.

Merck did a study in support of the registration of caspofungin.[111] Patients with invasive aspergillosis who had usually failed treatment with amphotericin B, lipid-based amphotericin B, or itraconazole, or who in a few cases were intolerant to amphotericin B, were treated with caspofungin alone (table 4). A good result (45% response rate) was obtained, showing that caspofungin is active in invasive aspergillosis.[111] Better results were seen in patients who were intolerant to amphotericin B than in those who failed treatment, as might be expected, and this small group of patients had a response rate of 75%. Since amphotericin B and itraconazole both have long half-lives, the response could effectively be a short period of combination therapy, with continued good effect of caspofungin. The other point to make about these studies is that many patients with invasive aspergillosis die very rapidly, and such patients are almost never enrolled into these trials because they do not survive long enough to be enrolled, or are excluded. Such studies therefore overestimate response rates compared with the general population of patients with the disease. The other point of note in the study was that response rates of patients with persistent profound neutropenia were not as good (<20%). This suboptimum response rate during persistent neutropenia accords with amphotericin B responses, although there are examples in published work of patients with longlasting neutropenia responding to itraconazole or voriconazole.[120,121]

A large study of micafungin in invasive aspergillosis has also been done (283 patients),[112,113] and a study of about 50 patients with various forms of aspergillosis in Japan.[114] In most patients, micafungin was added to existing treatment if the patient was not responding. Micafungin doses varied from 50 to 300 mg daily, and the investigator could increase the dose if the response was not thought to be adequate. Independent review of cases to establish diagnostic certainty and outcome, and inclusion of only those with confirmed invasive aspergillosis and at least 7 days of micafungin treatment, left 179 cases for analysis of response. The overall response rate was 37%, with a 40% response rate in the 35 patients treated with micafungin alone.[113]

Thus, the role of the echinocandins in the treatment of invasive aspergillosis is presently difficult to define. Good efficacy rates have been seen, and therefore they certainly represent an alternative for treatment when drug toxic effects are a problem or patients are failing therapy. Whether they will be superior to voriconazole as first-line therapy[122] is uncertain, and there are indications that they may be less effective in patients with neutropenia.

Fujisawa did a large prophylaxis study in support of their registration of micafungin.[115] In total, 882 patients

who received a haemopoietic stem-cell transplant were randomly allocated micafungin 50 mg/day or fluconazole 400 mg/day, for up to 6 weeks. Breakthrough infections, including those that arose up to 30 days after prophylaxis, were assessed. Endpoints included suspected fungal infection, and the treatment for this infection was amphotericin B. Micafungin response rates were better than those for fluconazole (80% *vs* 73·5%; p=0·025) and benefit was seen across all patient-groups, including children and elderly people, allogeneic and autologous transplant recipients, and patients with persistent neutropenia. Thus, despite the low dose of micafungin used, its benefit was clearly shown. The rate of documented aspergillosis was low. Whether the mortality benefits that have been seen with fluconazole[123] in this setting will be seen with micafungin in the future remains to be seen.

Combination therapy is an attractive option for patients with predictably poor clinical responses to present antifungal agents. This topic has been comprehensively reviewed for aspergillosis, the main focus of interest with respect to the echinocandins.[124] Antagonism has not been shown in vitro or in vivo, which is important. One retrospective assessment of the addition of caspofungin to amphotericin B in patients with cancer with invasive aspergillosis was encouraging,[125] as was the combination of itraconazole and caspofungin in two patients.[126] The response rates in patients on combination treatment were worse than for those on monotherapy with micafungin.[113] This finding probably indicates the actual or anticipated condition of the patient, and shows the need for studies to assess whether combination therapy is indeed advantageous. Combination treatment might be appropriate for very ill patients with predictably poor outcomes.

Adverse events and toxic effects

The adverse events and toxic effects of the echinocandins have been few. The maximum tolerated dose of caspofungin in rats was less than 38 mg/kg;[33] this value is not available for micafungin or anidulafungin.

Class-related toxic effects are shown in the panel. Histamine release is a frequent biological effect for basic polypeptide compounds. In animals treated with moderate and high doses of echinocandins given as an intravenous bolus, some evidence of histamine release was seen. For caspofungin, the no-observed-effect concentration with respect to histamine-like reactions was 2 mg/kg in rats and 8 mg/kg in monkeys with the clinical formulation.[33] For micafungin, this concentration was 10 mg/kg in rats, but infusion duration is a major determinant of this reaction and was not standardised in different laboratories. In patients, histamine-like reactions (not necessarily caused by histamine release) were not seen with any frequency after administration of caspofungin or micafungin, but could arise after anidulafungin if given too fast.

Adverse effects of the echinocandins

Class effects

- Headache
- Fever
- Liver toxic effects
- Phlebitis (caspofungin and preclinical)
- Histamine release
- Haemolysis

Other effects

- Rash

Local irritation at the infusion site is a problem for all compounds in preclinical studies. This sign has been noted in patients receiving caspofungin (about 20% incidence) but not micafungin. Liver toxic effects were manifest in several ways after administration of echinocandins. Doses of caspofungin of more than 2 mg/kg in monkeys caused rises in amount of alanine aminotransferase,[33] with a no-observed-effect concentration of 1·5 mg/kg. Larger doses (5 and 8 mg/kg daily) of caspofungin in monkeys led to patchy hepatic necrosis. Abnormal liver-function tests in patients receiving caspofungin were frequent, but not always associated with treatment, because the patients typically had other reasons for these abnormalities.[33,109] A high frequency of abnormal liver-function tests was seen with concurrent ciclosporin administration in early studies,[33] which increases exposure to caspofungin, suggesting that the therapeutic margin is small, although the clinical significance of this finding is unclear. Likewise, patients with micafungin also had abnormal liver-function tests (with a lower frequency than with caspofungin), but the relation to micafungin therapy was unclear in most instances.[112]

Haemolysis was seen in vitro and in some animals treated with echinocandins. Clinically significant haemolytic anaemia seems to be rare in clinical studies. Fever is a frequent side-effect of caspofungin treatment (arises in about 35% of patients).[33,103–105] Fever is uncommon with micafungin (about 1%).[112,119] Rash is an infrequent problem (<5%)—aside from flushing associated with a histamine-like reaction—with all three compounds. The combination of itraconazole and caspofungin might be more likely to lead to rash.[33] Headache is a frequent side-effect with all three compounds (3% with micafungin, about 15% with caspofungin).

Thus the toxic-effects profile of the three echinocandins is favourable, and certainly less of a problem than for amphotericin B, whether in complex with lipid or not. Caspofungin might have a narrower therapeutic window with respect to liver-function tests and concurrent use with ciclosporin than micafungin and possibly anidulafungin. Since the pharmacokinetics are predictable in different populations, unexpected dose-related toxic effects are unlikely. Idiosyncratic toxic events have yet to be described, and hopefully will not emerge, but vigilance is called for in view of the fact that this is a new class of chemistry now in human use.

Conflict of interest statement

In the past 5 years, D Denning has received grant support from Versicor, Fujisawa, Merck, Pfizer, Oxford Glycosciences, Novartis, F2G, Sequus, OrthoBiotech, Wellcome Trust, the Fungal Research Trust, the US National Institute of Allergy and Infectious Diseases, and the European Union; he has been a consultant to Merck, Fujisawa (presently for compilation, review, and revision of a submission to the European Medicine's Evaluation Agency for micafungin), Versicor, Oxford Glycosciences, Ranbaxy (unpaid), AstraZeneca, DSM Gist, PPL Therapeutics, Aventis, and GlaxoWellcome; and he has been paid for talks on behalf of Gilead, Merck, Fujisawa, Elan, and Janssen. He is a founder and minority shareholder in F2G.

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