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Department of Pharmaceutical Microbiology, Medical University of Lublin

ANNA SEREFKO, RENATA ŁOŚ, ANNA MALM

# *New drugs in antifungal therapy*

Both mucosal and invasive fungal infections have increased dramatically over the past several years and threaten the lives of patients who are immunocompromised due to cancer chemotherapy, organ or bone marrow transplantation or HIV infection. Current treatment strategies for these infections reveal important limitations, including antifungal resistance, toxicity, drug interactions and expense. Therefore the medical world is in dire need of more effective and safe alternative treatment to conventional therapy. New antimicrobial compounds in the class of triazoles and echinocandins that are currently in various stages of clinical development, appear to be promising options in treatment of serious, life-threatening fungal infections (14).

## TRIAZOLES

The second generation of antifungal triazoles includes voriconazole, posaconazole and ravuconazole. Currently only voriconazole has been recently introduced into antifungal therapy in Poland, the others are in various stages of clinical development. Voriconazole and ravuconazole are structurally closely related to fluconazole, whereas posaconazole structure is very similar to that of itraconazole (8).

The newest generation of triazoles acts by the same mechanism of action as their predecessors, namely by inhibition of fungal cytochrome P450-dependent 14α-lanosterol demethylase which is responsible for the conversion of lanosterol into ergosterol, a major component of the fungal cell membrane. It leads to accumulation of ergosterol precursors and inhibition of fungal growth (6).

All of the new azoles have an excellent *in vitro* activity against a wide variety of clinically important fungal pathogens, including *Candida spp.* (*C. glabrata* appears to be the least susceptible one), *Trichosporon beigelii*, *Cryptococcus neoformans*, *Aspergillus spp.* and several less common filamentous fungi, dermatophytes, most of hyaline moulds and dematiaceous as well as dimorphic fungi (6, 8). As for *Scedosporium* strains the antifungal potency of the novel triazoles varies according to the species. Ravuconazole, posaconazole and voriconazole display remarkable activity against *Scedosporium apiospermium* while *Scedosporium prolificans* isolates are resistant. Organisms within the *Zygomycetes* order are generally susceptible to posaconazole, whereas most of them are resistant to voriconazole and ravuconazole. It is notable that posaconazole possess both *in vitro* and *in vivo* potent antiparasitic activity against *Trypanosoma cruzei*, the causative agent of Chagas' disease. Ravuconazole and voriconazole are attractive candidates for the treatment of *Chaetomium* infections, for which there is no effective therapy nowadays, since they appeared to be *in vitro* efficacious against the pathogen mentioned (7, 10, 13).

In general, azoles are considered to be fungistatic. Nevertheless, the newer representatives of the class exert species-and strain-dependent fungicidal influence, e.g. against *Aspergillus spp.*, *C. neoformans* and certain non-albicans *Candida spp.* isolates (8).

The combination of voriconazole or posaconazole with polymorphonuclear leukocytes displays synergistic or additive activity against *S. prolificans* and *S. apiospermium* hyphae. Similar effects are observed for voriconazole and polymorphnuclear leukocytes against *Aspergillus fumigatus* (7). In the presence of serum voriconazole and ravuconazole exhibit concentration-dependent postantifungal effect (PAE) on *Candida albicans* (8).

Azole-resistant strains of *Candida spp.* usually display multiple mechanism of resistance. The most frequent appears to be: upregulation of efflux pump caused by overexpression of *CDR* and *MDR* genes, alteration of the fungal cytochrome P450-dependent 14α-lanosterol demethylase as a result of point mutation in the gene that encodes it (*ERG11*) and overexpression of *ERG11* gene. Cross-resistance between the old and newer azoles has been described (8).

As might be expected from structural similarity, ravuconazole and voriconazole should have the same susceptibility patterns, likewise posaconazole and itraconazole, although posaconazole is consistently more active. However, individual divergences of minimal inhibitory concentrations (MICs) may occur. A number of studies have reported that fluconazole -and itraconazole-resistant (the RR phenotypes) *Candida* isolates are significantly less susceptible to the novel triazoles in comparison to those that display resistance to fluconazole only (the RS phenotypes). Elevated itraconazole MICs against *A. fumigatus* are not usually associated with reduced activities of voriconazole or ravuconazole and may induce only a slight increase in posaconazole MICs. The laboratory-generated posaconazole-resistant *A. fumigatus* isolates exhibit cross-resistance to itraconazole but not to voriconazole. Similarly, *A. fumigatus* isolates resistant to voriconazole remain susceptible to posaconazole (8, 11).

All of the second generation triazoles have excellent bioavaibility after oral administration. Voriconazole and ravuconazole are also available in parenteral form. They undergo hepatic metabolism and are inhibitors of CYP3A4 isoenzyme (voriconazole additionaly inhibits CYP2C9/19). As such, they have the potential for numerous drug-drug interactions. Coadministration of voriconazole with long-acting barbiturates, carbamazepine, sirolimus, terfenadine, rifampin, rifabutin, astemizol, cisaprid, pimozide, qunidine and ergot alkaloids is contraindicated. Concomitant use of voriconazole with vinca alkaloids should be careful. Patients treated with certain agents (e.g. cyclosporine A, tacrolimus, HMG-CoA reductase inhibitors) and voriconazole at the same time require being closely monitored (6, 14).

Voriconazole has been licensed for primary treatment of acute invasive aspergillosis, flukonazole-resistant serious invasive *Candida* infections and as a salvage therapy for rare but serious fungal infections caused by *S. apiospermium* and members of the genus *Fusarium*. Interestingly, despite poor *in vitro* activity against *Fusarium spp.*, a high number of patients with fusariousis have responded well to both voriconazole and posaconazole treatment. Voriconazole has also proved to be a potent new weapon for empirical therapy in patients with neutropenia and persistent fever (14).

As for posaconazole, it is being developed for the treatment of life-threatening invasive fungal infections that are refractory to currently available antifungals. So far, it has demonstrated clinical efficacy and usefulness in the management of invasive zygomycosis, pulmonary histoplasmosis, chromoblastomycosis, mycetoma, filamentous fungal infections, and refractory central nervous system infections due to cryptococcosis. Ravuconazole treatment of invasinve cryptococcosis and histoplasmosis has been effective in experimental animal models (8, 15).

The novel azole compounds are generally well tolerated. The majority of the adverse events are non-specific and of short duration. Worth noting are reversible transient visual disturbances (blurred vision, photophobia, color vision changes) typical of voriconazole therapy. These visual abnormalities are said to follow the rule of 30 s: they are observed in around 30% of patients receiving voriconazole, start about 30 min after drug administration and last about 30 min (6, 14).

## ECHINOCANDINS

Amongst all representatives of the echinocandin class, there are three compounds particularly worth mentioning: caspofungin which has been licensed in the USA and in Europe, also in Poland, and anidulafungin and micafungin that undergo the advanced clinical trials. They are water-soluble synthetically modified derivatives of the natural lipopeptides, fermentation products of various fungi (3). Structurally, they are cyclic hexapeptides N-linked to a fatty acyl side chain. Their antifungal activity is conditioned by the covalent bond between the peptid core and lipophilic side

chain. Separation of these two parts of the molecule or replacement of chemical bond with a non-covalent one results in a loss of antimicrobial properties of echinocandins (4, 12).

Echinocandins are non-competitive inhibitors of the (1,3)-β-D-glucan synthase complex which catalyzes the polymerization of UDP-glucose into (1,3)-β-D-glucan, an essential component of the cell wall of numerous fungal species. The chains of this homopolysaccharide are predominantly responsible for controlling the internal turgor pressure of the cell and maintaining its integrity and rigidity. Besides, they play an important role in the cell growth and its division (1, 4).

Theoretically, echinocandins should be active against all fungi that possess (1,3)-β-D-glucan. However, numerous *in vitro* and *in vivo* studies have narrowed down this assumed spectrum of activity. All of echinocandins exhibit potent activity against yeasts of the genus *Candida*, including isolates resistant to azoles and amphotericin B (worthy of note are relatively high MICs required by *C. parapsilosis* and *C. quilliermondii* strains) and against filamentous fungi of *Aspergillus* spp. Dimorphic fungi are susceptible to echinocandins in differential manner. Interestingly, micafungin efficacy depends considerably on the growth form of these pathogens. The drug is significantly *in vitro* active against the mycelial forms of *Histoplasma capsulatum*, *Blastomyces dermatitidis* and *Coccidioides immitis*, whereas their yeast-like forms seem to be resistant to micafungin. Similar pattern of susceptibility profile is characteristic of *Pneumocystis carinii* isolates. Only the cyst form of this pathogen contains (1,3)-β-D-glucan in its cell wall and is susceptible to echinocandins. However, a few reports have indicated echinocandins activity against the trophozoite form as well. *C. neoformans*, *Rhizopus arrhizus*, zygomycetes and fungi of the genus *Fusarium* and *Trichosporon* are resistant to the representatives of the echinocandin family. *S. apiospermium* is susceptible to caspofungin but resistant to both micafungin and anidulafungin (3, 4).

*In vitro* studies have demonstrated that echinocandins are fungicidal against *Candida* spp. isolates. Inhibition of (1,3)-β-D-glucan synthesis leads to disruption of cell wall structure and brings about osmotic instability and eventually results in death of the microorganism. As for *Aspergillus spp.*, the situation is quite different. Echinocandins exert their fungistatic influence by selective destruction of growing branching points and apical cells of the hyphae leading to the formation of flattening and swelling tips and hyphal fragmentation. These damaged cells possess distinctly reduced angioinvasive potential. The effect on hyphal damage is concentration-dependent, thus the minimal effective concentration (MEC), a novel measure of susceptibility to echinocandins, defined as the lowest concentration able to produce the morphological alterations, has been introduced (3, 4).

Of particular importance is the echinocandins potency against preformed *Candida* biofilms. Although they do not eradicate biofilm thorougly, they kill the vast majority of sessile cells. Preincubation of planktonic *C. albicans* cells with subinhibitory concentrations of these antifungal agents reduces their ability of subsequent biofilm formation (9).

Oddly enough, enhancement of echinocandins efficacy against *Aspergillus* spp. in the presence of human serum has been reported. Similarly, escalation of inhibitory activity against *Aspergillus spp.* hyphal growth is observed for caspofungin cocultured with human monocytes and macrophages (4).

Resistance to echinocandins is primarily associated with mutations of the *FKS* and *RHO1* genes that encode a catalytic and regulatory subunit of glucan synthase respectively. However, the overexpression of *SBE2* gene that encodes the Golgi protein involved in the transport of cell wall components, results in decreased susceptibility to echinocandins. It is still matter of argument whether the compounds that belong to the echinocandin class are the substrates for multidrug transporters. However, several reports indicate that the ABC transporter encoded by *CDR2* gene confers resistance to caspofungin when constitutively overexpressed (4). *C. neoformans* and *H. capsulatum* have developed a very interesting mechanism of resistance to antifungal agents, including caspofungin. These fungal pathogens produce melanin-like pigments that bind the drug and inhibit its activity (5). It is worth mentioning that the lack of cross-resistance between echinocandins and amphotericin B or azoles is observed (4).

Owing to minimal absorption after oral administration, caspofungin, micafungin and anidulafungin are only available for intravenous use. Their favourable pharmacokinetic properties permit

once daily dosing. Echinocandins are considered to be not metabolized through the cytochrome P450 enzyme. However, clinical trials have proved that the coadministration of caspofungin with inducers or mixed inducer/inhibitors of CYP450 isozymes, such as efavirenz, nelfinavir, nevirapine, phenytoin, rifampine, dexamethasone and carbamazepine may reduce caspofungin concentration. Concomitant administration of caspofungin and cyclosporine is not recommended due to an increased area under the curve of caspofungin and may lead to liver problems. Tacrolimus concentration is reduced when coadministered with caspofungin (3, 4, 14).

The animal studies have revealed that a deleterious drug interaction occurs between anidulafungin and glucocorticoids, such as cortisone, hydrocortisone and triamcinolon, with the exception of dexamethasone (2). The two-drug combinations of azoles, amphotericin B, polyenes and nikkomycin with the echinocandin compounds frequently show a synergistic or additive activity against fungal pathogens refractory to monotherapy. No antagonism has been detected so far (1, 4).

Caspofungin is highly effective as a salvage therapy for the treatment of mucosal and invasive candidiasis and systemic aspergillosis in immunosuppressed patients refractory to or intolerant of conventional antifungal agents. The excellent safety profile and broad spectrum of activity suggest a possible role for caspfungin and other echinocandins in empirical therapy. Micafungin and anidulafungin have not been approved by Food and Drug Administration (FDA) yet. A number of experimental studies of *P. carinii* infections, systemic candidiasis and aspergillosis in normal and immunocompromised animals have displayed the efficacy of these both echinocandin B derivaties. Moreover, treatment with micafungin has given good clinical outcomes for HIV positive patients with esophageal candidiasis. Besides, micafungin has proven to be efficacious as the primary prophylaxis therapy for fungal infection in bone marrow transplant recipients (3, 4).

Because of the fact that mammalian cells are deprived of (1,3)-β-D-glucan by nature, the inhibitors of its synthesis possess excellent safety profile and are well tolerated by both human and animal organisms. The most common drug-related side-effects are fever, phlebitis at the infusion site, headache, nausea, skin rash and transient mild-to-moderate elevation of hepatic enzyme levels (1, 3, 4).

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SUMMARY

Fungal infections, particularly in immunosuppressed patients, have increased dramatically in recent decades. Growing problems of resistance and cross-resistance of yeasts and moulds pathogens to antimicotics, toxicity of antifungal agents, drug interactions, create a need for novel effective antifungal compounds. We present new antifungal agents belonging to echinocandins (caspofungin, micafungin and anidulafungin) and the second generation of triazoles (voriconazole, posaconazole and ravuconazole). Due to their pharmacokinetic properties, broad antimicotic spectrum, safety and tolerability profile these agents appear to be a useful addition to the antifungal armamentarium, especially in treatment of serious, life-threatening fungal infections.

Nowe leki przeciwgrzybicze

W ostatnich dziesięcioleciach gwałtownie wzrasta liczba zakażeń grzybiczych, co jest wynikiem powiększającej się populacji chorych o obniżonej odporności. Narastający problem oporności oraz oporności krzyżowej drożdżaków i grzybów pleśniowych na leki przeciwgrzybicze, toksyczność tych związków i ich interakcje z innymi lekami, uzasadniają potrzebę poszukiwania nowych antymikotyków. W pracy przedstawiono nowe leki przeciwgrzybicze należące do echinokandin (kaspofungina, mikafungina, anidulafungina) oraz drugiej generacji triazoli (worikonazol, posakonazol, rawukonazol), które ze względu na korzystne właściwości farmakokinetyczne, szerokie spektrum działania, bezpieczeństwo stosowania, wydają się obiecującą alternatywą dla obecnie stosowanych preparatów przeciwgrzybiczych, zwłaszcza w leczeniu ciężkich zakażeń grzybiczych.