Echinocandins Versus Dated Antifungals in Combination Against Opportunistic Mycotic Infections

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Received: 19 January 2017, Revised and Accepted: 03 February 2017

Abstract
Scientific and clinical reports globally demonstrated that the opportunistic mycotic infections are at major risk to the human fitness. In the past few decades, the development of resistance in microbes to existing antifungals has emphasized on the search of new antymycotic drugs. As a matter of fact, “echinocandins” are new categories of broad-spectrum antifungal that enlighten a hope in this direction. Echinocandins are bulky lipopeptides that inhibit the production of β-[1,3]-glucan “a major constituent of fungal cell wall” which ultimately leads to the death of fungal pathogens. In vitro as well as in vivo published reports have demonstrated that the echinocandins exhibit fungicidal activity against most Candida spp., while fungistatic against Aspergillus spp. and exclusively found to be more effective when tested in combination with polyenes/azoles. The present article is expert views on the recent and historical literature available on the antifungal therapies with accessing their impact on the human health. Emphasis is given to the utility of the echinocandins a potential antifungal agent by discussing recent examples of clinical and laboratory studies including the use of improved proteomics approaches to know a bit more about the interaction of human host and fungal pathogens.

Keywords: Echinocandins, Antifungal, Aspergillus, Candida, Opportunistic, Combination.

Introduction
A normal resident flora of the body also includes pathogenic microbes, which develop disease only when host immune defense is weak. Opportunistic mycoses are the type of fungal infections mostly occurring in the host with compromised immune system. Over the past few decades, the incidence of invasive fungal infections due to opportunistic pathogens has been escalated significantly [1]. This upsurge in infections is coupled with extreme morbidity/mortality [2], also the individuals undertaking blood/bone marrow transplantation, solid organ transplantation, and major surgery are at higher risk of such kind of infections [2,3]. Highest frequencies of opportunistic fungal infections are due to different species (spp.) of Candida (Candidiasis and Candidaemia) and Aspergillus (Aspergillosis). Current medical treatments are of limited utility, and their therapeutic use has been complicated by Candidaemia and invasive Candidiass (IC), which contribute toward high morbidity and mortality [4]. Candidaemia is the fourth major type of microbial bloodstream infection [5], while Candida albicans being the major causative spp. for the development of IC [6]. Over the past two decades, the prevalence of Candidaemia has boosted as per the hospital records, which has shown the incidence ranging from 24% to 57%. In a report of out of 670 cases of Candidaemia, 274 (41%) were hospital acquired, among which mortality turned out to be 39% [7] Both C. albicans and Candida non-albicans species are known to colonize the skin, gastrointestinal tract and reproductive tract in humans apart of Candidaemia, Invasive Aspergillosis (IA) which is caused by spp. of Aspergillus (Aspergillus fumigatus, Aspergillus flavus, Aspergillus terreus, and Aspergillus nidulans), has also been emerged as a major life risk in immunocompromised patients [8]. Recently, in an 18 month surveillance program, among 5,561 patients, only 12 patients were reported to be suffering from IA (0.2%). However, mortality was found to be 60% [9]. Furthermore, researchers affirmed 7% of IA affected cases by a mortality rate of 91%. Remarkably, 70% of these cases had been observed with no predisposing factors for opportunistic mycotic infections [10].

At present, major drugs used for systemic therapy of opportunistic fungal infections have different main targets: The polyenes and azoles affect the plasma membrane, whereas the antimetabolite, 5-fluorocytosine restricts DNA and RNA synthesis whereas echinocandins acts on the cell wall of the pathogens. Fluconazole targets lanosterol 14-alpha-demethylase and hinder the biosynthesis of ergosterol [11]. Since the first polyene was standardized 40 years earlier, the echinocandins were approved in January 2001 as a new class of antifungal drug. Different types of echinocandins include micafungin (MCF) (Mycamine, Astellas) which was accepted in 2005 and anidulafungin (ANF) (VOR-002, V-echinocandin, LY303366, Vicuron Pharmaceuticals) accepted in 2006; further, these two classes of echinocandin have been approved by the federal drug administration [12]. Since 2000, echinocandins have been extensively used for the cure and prophylaxis of invasive mycoses (IM) [13] and majorly suggested as first-line treatment for Candidiasis [14].

Echinocandin reveal effective antifungal activity against major infectious fungi, such as Candida spp., Aspergillus spp., and Pneumocystis carinii. However, in case of Cryptococcus neoformans, current echinocandins lack in vitro activity. Although, semisynthetic echinocandins are on more beneficial sides like favorable pharmacokinetics allowing once-daily administration low toxicity and fast antifungal activity. The echinocandins lately accessible for clinical purposes are caspofungin (CAS), MCF, and ANF [15]. Results of clinical practices applied in China suggest that CAS has been superior to the other two antifungal drugs classes: Polyenes and azoles, because of its efficiency in curing fungal infections (15% superior to fluconazole); minor adverse effects such as hepatic dysfunction, infusion-related reaction and vomiting (25-50% lower frequency); rapid resolution of symptoms (almost 3 days faster than Amphotericin B [AmpB]); and no antagonism in combination therapy [16].

Constant efforts are needed to develop the suboptimal remedial results linked with mycotic infections. However, monotherapy is often diagnosed with increased risk of toxicity, lesser resistance, or limited spectrum of activity. These limitations of monotherapy led to determine efficacy of combination therapy for the treatment and management of IM [17]. There are various probable benefits to combination antifungal therapy, i.e., wide spectrum and effectiveness of drug activity, speed antifungal effect, synergy, limited dose, and chance of antifungal
resistance is reduced. Although every antifungal agent has its own restrictions, therefore, combination therapy might become more efficient for the treatment of IM [18].

In this article, we have emphasized on echinocandins being potential source as an antifungal agent, their improved efficacy in combination with currently available antifungals including pharmacokinetics investigations have been discussed along with available reports on genomics and proteomics based studies, for elucidating the mechanism of action of these antifungal drugs.

**ECHINOCANDINS: THE POTENTIAL SOURCE AS AN ANTIFUNGAL AGENT**

Recently, only a few antifungals are in use that has significant efficacy against fungal infections. These antifungal drugs affect specifically the components constituting cell membrane of fungi or its biosynthetic pathways. However, more recent class of antifungals in use is echinocandins [19], which disrupt cell wall components by non-competitively inhibiting the synthesis of 1,3-β-glucans. First reported echinocandin was CAS, followed by MCF and ANF. CAS is found to be most effective against candidal esophagitis and candidemia, along with salvage therapy of Aspergillus infections and for empirical therapy of febrile neutropenia. Likewise, MCF is used in treating candidal esophagitis and cases of hematopoietic stem cell transplants. ANF has also been considered an effective drug to cure candidal esophagitis, Candidaemia [20].

Echinocandins seems the smart new choice for the cure of IM infections because of their reduced toxicity and slight drug-drug interactions. They are found to be fungicidal against yeast and fungistatic against mold. However, their price may reduce their usage during initial therapy in cases with fungemia in medical centers or ICU with a high rate of triazole-resistant Candida infections. Reportedly, Echinocandins act fungicidal against Candida spp. along with triazole-resistant isolates and show fungistatic activities against Aspergillus spp. echinocandins also reveal concentration-dependent activity against Candida spp. Although echinocandins are fungistatic against mold, according to one study, they might prove promising for treating opportunistic infections when administered in combination with AmpB or wide-spectrum triazoles, such as voriconazole. Reviews suggest that in clinical trials, CAS exhibit efficacy in curing esophageal candidiasis, candidemia, and febrile neutropenia. However, MCF seems effective in hematopoietic stem cell transplant recipients and the treatment of esophageal candidiasis. ANF received approved labeling from the Food and Drug Administration in February 2006 [21].

Since 2000s, echinocandins are extensively recommended for the treatment of invasive fungal infections, remarkably, and 1C. Although, cases of advance candidiasis in hosts, taking echinocandins, have been detected; however, the rare incidence of clinical failure is observed due to the possession of resistance by a generally susceptible Candida spp. isolate [13]. Reportedly, *in vitro* activity of antifungal drugs was tested against 496 isolates of yeasts and molds, during 2010-2012. Fks hot spots were sequenced for strains resistant to echinocandins and resistance was found infrequent among 6 Candida spp. and was only spotted in 3 isolates of Candida glabrata, having mutations in fks1 (F625S) and fks2 (S663P) [22].

Another study of 163 hosts during 2011-2012, revealed the fact that echinocandin failure was rare and molecular investigations of the fks1 and fks2 hotspots of the C. glabrata, discovered mutations only in 2 isolates (L628R and S629P in fks1) [23].

Furthermore, a report comprising data from 2008 to 2013 suggested that, among 1,380 hosts of C. glabrata, 3.1% were resistant to ANF, 3.3% were non-responsive to CAS and 3.6% were not affected while treated with MCF [24].

Mechanism of resistance

Echinocandin resistance in *C. glabrata* is associated entirely with fks1p and fks2p amino acid substitutions [25]. Echinocandin failure accredited to mutations in fks1 is linked with raised chitin levels and the lack of a compensatory upsurge in chitin level on echinocandin introduction (Table 1) [26].

**MONOTHERAPY/COMBINATION THERAPY OF ECHINOCANDINS AND THEIR CHEMICALLY MODIFIED DERIVATIVES**

**Echinocandins in combination with polyenes**

Recently, combination antifungal therapy of echinocandins with polyenes has been explore in nonclinical studies for boosting the effect of the treatment for IA. AmpB aims at ergosterol present in fungal cell membrane by binding with it consequently creating pores in the membrane and disrupting membrane integrity, resulting in fungal cell lysis [27]. However, the echinocandins mainly focus the enzyme 1,3-β-D-glucan (BG) synthetase, required for the synthesis of L3-BG, a vital component of fungal cell wall [28]. The specificity of the echinocandins against the fungal cell wall also seems promising for least hazardous side effects. Hence, combination of a drug, which aims at the cell wall and one, targeting the plasma membrane, might result in additive or synergistic antifungal effects. Nonclinical reports suggest that the combination of AmpB and the echinocandin does not act antagonistic during the treatment of Aspergillus infections [29].

Indeed, enhanced efficiency has been displayed by mice model (affected with chronic granulomatous disease and pulmonary IA) when administrated with combination of MCF (Mycamine) and AmpB [30] and in case of murine systemic aspergillosis, increased efficacy has been reported when treated with a combination of CAS plus AmpB or intra-lipid AmpB (LAmPB) [31]. Whereas, Clemons and Stevens [29] reported that there was no synergistic activity of MCF and AmpB for pulmonary IA in immunocompromised DBA/2 mice, although there was no antagonism too.

In another case of a Systemic Murine Aspergillosis model [32], the researchers observed narrow additive effects of suboptimal doses of LAmPB and MCF with appreciably decreased fungal load in the spleens when treated with LAmPB before MCF. Although for the disseminated and pulmonary murine infections, administering LAmPB along with MCF or CAS, was reported to be neither antagonistic or additive nor synergistic although improved survival or decrease in fungal load also considered. However, Wassen et al. [33] observed additive effects in a rat model (infected with *A. fumigatus*) when it was exposed LAmPB (5 mg/kg) plus CAS (3 mg/kg), parallelly. In this study, the researchers reported that the combination appreciably reduced colony forming unit (CFU) (by 98%), while LAmPB or CAS monotherapy reduced 69% and 80% CFU, respectively, in comparison with untreated controls.

Instead of combination therapy, researchers analyzed the decline of fungal load in kidneys during disseminated infection, was appreciably improved when LAmPB was administered previous to CAS instead of CAS was given first (sequential therapy) [34].

In conclusion, treatment of disseminated or pulmonary infection of *A. fumigatus* in mice, with LAmPB + echinocandin or exposure of LAmPB previous to the echinocandins, was equally efficient as LAmPB treatment alone, but with prolonged survival and reduced fungal load in the target tissues. In contrast, unlike pulmonary infection, in the hosts of disseminated infection, survival was prolonged by echinocandin monotherapy, and in general, did not considerably decrease the fungal load compared to LAmPB. Taken both models into account, there was appreciably improved effect when LAmPB was given earlier to the echinocandins; these explanations deliver a convincing evidence for using LAmPB first if sequential therapy is used for IA [35].

**Echinocandins in combination with azoles**

A research, including 101 cases (79 proven, 22 probable), across four risk groups (patients with hematologic malignancy, stem cell recipients,
Drugs Act as scavenger for Flucytosine voriconazole with ANF exhibited greater survival in patients with IA [38]. as compared to single drug administration, combination therapy of monotherapy, it was, 27.5% (39 of 142) (p=0.087); concluding that rates observed at 6 weeks, was 19.3% (26 of 135) and for voriconazole randomly given voriconazole and ANF. For combination therapy, mortality hematopoietic stem cell transplantation and proven or probable IA, were 93 international sites, 454 patients with hematologic malignancies or (RR=0.47) [37].

In a more recent study involving 10 randomized controlled trials, involving 2,837 hosts, echinocandins and triazoles exhibited parallel outcomes in terms of promising treatment success rate such that the relative risk (RR)found to be 1.02, fungal success rate with RR 0.98, advance infection RR to be 1.09, drug-associated adversity with RR 0.94, and all-cause mortality (RR=0.85; 95% cumulative prevalence, 0.66-1.10). Moreover, echinocandins were found to be more efficient than triazoles in case of patients diagnosed with hematologic malignancies or hematopoietic stem cell transplantation recipients with RR of 1.08. Echinocandins appreciably reduced the adverse effects linked to the withdrawal rate in comparison with triazoles (RR=0.47) [37].

In another research, it was found that combination therapy (MCF and daily dose of oral 400 mg itraconazole) can be promising alternative for treatment of fatal IA of the sinus [39].

A study conducted in Germany from 2006 to 2012 in patients with acute alternative lymphoblastoma leukemia and hematopoietic stem cell transplantation recipients having IM. Out of 25 patients of acute lymphoblastoma leukemia along with 28 cases of suspected IM, 20 were administered with empirical CAS first line monotherapy (71.4%). 5 were given second line monotherapy (17.9%) and combination therapy in 3 cases (10.7%) concluding that empirical CAS seems to be a successful therapeutic alternative in case of IM having acute lymphoblastoma leukemia with probable IM [40].

In an observational study conducted in Japanese patients, from July 2007 to June 2010, it was observed that among 241 patients, 86 patients (35.7%), were diagnosed with 143 adverse drug reactions, especially hepatobiliary disorders. The clinical success rate was 72.8% (131/180 patients), and the prevalence of advance infections was merely 4.4% (8/180 patients) concluding that MCF had satisfactory efficiency against IM in Japanese episodes undergoing hematopoietic stem cell transplantation [41].

In a randomized, double-blind, placebo-controlled, multicenter trial from 93 international sites, 454 patients with hematologic malignancies or hematopoietic stem cell transplantation and proven or probable IA, were randomly given voriconazole and ANF. For combination therapy, mortality rates observed at 6 weeks, was 19.3% (26 of 135) and for voriconazole monotherapy, it was, 27.5% (39 of 142) (p=0.087); concluding that as compared to single drug administration, combination therapy of voriconazole with ANF exhibited greater survival in patients with IA [38].

The relative risk (RR) found to be 1.02, fungal success rate with RR 0.98, advance infection RR to be 1.09, drug-associated adversity with RR 0.94, and all-cause mortality (RR=0.85; 95% cumulative prevalence, 0.66-1.10). Moreover, echinocandins were found to be more efficient than triazoles in case of patients diagnosed with hematologic malignancies or hematopoietic stem cell transplantation recipients with RR of 1.08. Echinocandins appreciably reduced the adverse effects linked to the withdrawal rate in comparison with triazoles (RR=0.47) [37].

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Combination therapy of pediatric IM has hardly been reported. In a recent study, conducted on 19 children (with a median age of 5.3) affected with IM, were administered with liposomal LamPb monotherapy for a median duration of 12 days (range 3-69 days). As patients were obstinate to LamPb; therefore, CAS was supplemented in 11 patients. In the remaining 6 cases, LamPb was stopped and a combination of CAS and voriconazole were commenced. 12-week survival rate of these patients was 75% with no hazardous side effect. Data suggest that combination antifungal therapy is safe and effective in children with hematological malignancies [36].

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Present recommendations acclaim antifungal measures for children who are at high risk for IM, though the administration of polyenes and triazoles may not be recommended in some episodes due to toxicities and drug-drug interactions. Although Azole antifungal drugs are administered clinically in patients with IM, these agents have been detected with renal toxicity thus causing vomiting. Thus it has led to requirement of new antifungal agents with minimum toxicities and drug-drug interactions. Although Azole antifungal drugs are administered clinically in patients with IM, these agents have been detected with renal toxicity thus causing vomiting. Thus it has led to requirement of new antifungal agents with minimum toxicities and drug-drug interactions. Although Azole antifungal drugs are administered clinically in patients with IM, these agents have been detected with renal toxicity thus causing vomiting. Thus it has led to requirement of new antifungal agents with minimum toxicities and drug-drug interactions. Although Azole antifungal drugs are administered clinically in patients with IM, these agents have been detected with renal toxicity thus causing vomiting. Thus it has led to requirement of new antifungal agents with minimum toxicities and drug-drug interactions. Although Azole antifungal drugs are administered clinically in patients with IM, these agents have been detected with renal toxicity thus causing vomiting. Thus it has led to requirement of new antifungal agents with minimum toxicities and drug-drug interactions. Although Azole antifungal drugs are administered clinically in patients with IM, these agents have been detected with renal toxicity thus causing vomiting. Thus it has led to requirement of new antifungal agents with minimum toxicities and drug-drug interactions. Although Azole antifungal drugs are administered clinically in patients with IM, these agents have been detected with renal toxicity thus causing vomiting. Thus it has led to requirement of new antifungal agents with minimum toxicities and drug-drug interactions. Although Azole antifungal drugs are administered clinically in patients with IM, these agents have been detected with renal toxicity thus causing vomiting. Thus it has led to requirement of new antifungal agents with minimum toxicities and drug-drug interactions. Although Azole antifungal drugs are administered clinically in patients with IM, these agents have been detected with renal toxicity thus causing vomiting. Thus it has led to requirement of new antifungal agents with minimum toxicities and drug-drug interactions. Although Azole antifungal drugs are administered clinically in patients with IM, these agents have been detected with renal toxicity thus causing vomiting. Thus it has led to requirement of new antifungal agents with minimum toxicities and drug-drug interactions. Although Azole antifungal drugs are administered clinically in patients with IM, these agents have been detected with renal toxicity thus causing vomiting. Thus it has led to requirement of new antifungal agents with minimum toxicities and drug-drug interactions. Although Azole antifungal drugs are administered clinically in patients with IM, these agents have been detected with renal toxicity thus causing vomiting.
spp. biofilms. These findings may suggest new therapeutic strategies of ANF with NSAIDs against Candida biofilm-related IM [44].

Echinocandin in combination with adjunctive agents

Hematopoietic growth factors encourage the proliferation and stimulation of phagocytic host cells; out of these, colony-stimulating factor (G-CSF), GM-CSF, and M-CSF have been considered as adjunctive immunomodulatory agents against fungal pathogens.

G-CSF incites the production and differentiation of myeloid progenitor cells to polymorph nuclei leukocytes, such that raising the quantity of mature neutrophils. Moreover, In vitro G-CSF boosts the phagocytic activity of neutrophils along with respiratory burst against Aspergillus spp. [44, 45]. In neutropenic animal models of IA, G-CSF treatment was coupled with improved survival as well as quicker recovery from neutropenia [46].

ADVANCE THERAPIES FOR BETTER TREATMENT STRATEGIES AND NEED FOR NOVEL DRUG TARGET IDENTIFICATION

As most of the pathogenic spp. of Candida and Aspergillus has developed resistance against standard antifungal drugs, there is a need to search advance therapies for better treatment of IM. Recently, a novel echinocandin-type antifungal metabolite, MIG0310, with a molecular formula C(48)H(66)O(18) was isolated and characterized, obtained originally from fungal strain, Fusarium MS-R1, has led to the idea of developing new antifungal drugs against resistant Candida spp. [47].

Another interesting research showed that an inhibitor of Hos2 fungal histone acetylase named as MGCD290 employs a noticeably satisfactory impact on the minimum inhibitory concentrations (MICs) of fluconazole and the echinocandins, showing synergy when administered in combination, converting resistant Candida spp. to susceptible, irrespective of fks mutations [48].

In 2015, Wiederhold et al. [49] confirmed in vitro and in vivo antifungal activity of arylamide, T-2307 against C. albicans, resistant to echinocandin along with usefulness of the unirid echinocandin ASP9726 in a guinea pig model with IA. Guinea pigs were subcutaneously injected with investigational echinocandin at different concentrations, and plasma concentrations were noted. Immunocompromised guinea pigs were infected with A. fumigatus and three drugs (ASP9726, voriconazole, CAS) were given for 8 days. Measurement of variations in fungal burden showed that ASP9726 plasma concentrations were raised proportionally with every dose, and the drug was well tolerated at every dosage. Every dosage of ASP9726 at 5 mg/kg had considerably improved survival [50].

Current epidemiological investigations have disclosed rise in number of Aspergillus spp. biofilms. These findings may suggest new therapeutic strategies of ANF with NSAIDs against Candida biofilm-related IM [44].

In a study comparing the echinocandins activity against 7 C. albicans, 5 C. dubliniensis, and 2 C. africana spp. by time-kill analysis, MIC values were found to be similar for the 3 spp. as echinocandins displayed weak killing activity and no mortality against C. africana. This study suggests time - kill analysis can be taken as a promising diagnostic tool for identification of new pathogenic spp. especially when echinocandin therapy collapses [55].

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strain [fks1-S67BP] was taken to authenticate proteins unambiguous to drug action. Also, Microarray analysis of non-resistant strain has been accomplished for assessment of the link that associates proteomics with genomics such that overall 117 genes were discovered which vary minimum by 2 times. However, 22 proteins with noteworthy deviations were acknowledged by iTraq also displayed substantial alterations in their level of gene expression analyzed by microarray [61].

Recent studies used the combination of matrix-assisted laser desorption ionization-time of flight mass spectroscopy and two-dimensional polyacrylamide gel electrophoresis to recognize the modifications in protein richness in a strain of C. albicans following treatment with CAS. Mainly the proteins involved in glycolysis and gluconeogenesis gave response to CAS exposure, while other proteins reported to respond specifically to this drug found to be involved in cell stress and heat shock, and the proteins of the Kreb’s cycle and amino acid biosynthesis. Some enzymes catalyzing cell wall biosynthesis and the regulating BG synthase were also acknowledged [62].

FUTURE PROSPECTIVE

The medical practice with echinocandin drugs has been highly successful, as this class of drugs exhibit strong efficacy, especially with Candida spp., with negligible side effects and a low frequency of resistance. In future, its effect in combination with chelators can be explored. Moreover, it might give significant results with adjunctive immunotherapeutic agents under which research is under dogged. Echinocandins can be promising for novel drug design and might prove a milestone for the pharmaceutical industry in future.

ACKNOWLEDGMENT

The authors would like to thank Maharishi Dayanand University for necessary support.

REFERENCES


