Invasive aspergillosis: adjunctive combination therapy

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Abstract: Invasive aspergillosis remains a serious opportunistic fungal infection particularly in patients with a reduced immune defense such as those with hematological malignancies or transplant recipients. The mortality of invasive infections due to *Aspergillus* spp. is still high. The main reasons for this are the difficulty in diagnosing of these infections and the limited efficacy of antifungal agents. There is no optimal therapy for invasive aspergillosis, and therefore many clinicians have attempted to utilize a combination approach to improve outcomes. The current antifungal classes of drugs targeting the cell wall and cell membrane may need adjunctive agents focused on separate cellular pathways that can be used in combination therapy to maximize the efficacy, a valuable alternative to the monotherapy. The endeavor of this article is to review the literature on combination therapy by using adjunctive agents against *Aspergillus* spp and assess its eventual usability in the treatment of invasive aspergillosis.

Keywords: *Aspergillus*, Aspergillosis, Adjunctive agent, Combination therapy, Monotherapy.

INTRODUCTION

The number of invasive fungal disease has dramatically increased over the past few decades corresponding to the rising number of immunocompromised patients. The clinical consequences of antifungal resistance & poor host functions are evident in treatment failures as well as in the changing prevalence of fungi. The aspergillosis related mortality in acute myeloid leukemia is 30-40 % [1] & number of patients with aspergillosis in the US ranges from 0.5% (after autologous hematopoietic stem cell transplantation) to 3.9% (after transplantation from an unrelated donor). In these patients after diagnosis of aspergillosis within three months mortality was 53.8% in autologous transplant recipients and 84.6% in those with unrelated donor transplants [2] According to the most recent data from the US, annual mortality rate from invasive aspergillosis (IA) was 25% [3]. This shows that there is improvement but the risk of fungal infection after transplantion is multifactorial. Literature describing antifungals used to prevent IA after transplant is scarce [4].

This shows that our current antifungal armamentarium is not adequate. Moreover, the development of antifungal drugs has lagged far behind that of antibacterial agents. Fungi are eukaryotes and despite the presence of a cell wall, fungi are more similar to mammalian cells on cellular level than to bacteria. Additionally, fungi replicate more slowly than bacteria and are often difficult to quantify, particularly for moulds, which complicates efficacy assessments. Currently drugs used for systemic therapy of invasive mycoses have three main targets: the polyenes and azoles target the cell membrane, the antimetabolite 5-fluorocytosine interferes with DNA and RNA synthesis and echinocandins affect the cell wall. In January 2001, the echinocandins (Caspofungin) became the first semisynthetic antifungal natural product to be approved since polyene was approved forty years earlier, and in 2005 micafungin (Mycamine, Astellas) became second and in 2006 Anidulafungin (VER-002, V-echinocandin, LY303366, Vicuron Pharmaceuticals) became third FDA approved echinocandin. The other class azole (except Voriconazole (2002) & Posaconazole (2006), allylamine & antimetabolite were present prior to 2001.

The new antifungal class echinocandin and some other drugs (adjunctive agents – exhibit inhibitory effect on pathogen except fungi) can increase the permutations of combination therapies for IA. Combination therapies for IA seem plausible to optimize the therapy. There are several foreseeable advantages of combination therapy like widened spectrum, potency of drug therapy, lowering the dose of toxic drugs, synergy and reduced risk of antifungal resistance. Utilizing the agents with different mechanism of action is hallmark in current medical therapies, but of course one has to be cautious as they may be antagonistic or clinically indifferent with additive side effects. It cannot be simply assumed that the use of two or more effective drugs with different mechanisms of action will produce an improved outcome, but the current antifungal approach is clearly not optimal as patients continue to die from IA.

Fractional inhibitory concentration (FIC) index (FICI) is most frequently used to define *in-vitro* drug interaction-synergy (SYN), antagonism, indifference (IND). SYN is a phenomenon in which two different compounds are combined to enhance their individual activity where as if combination results to a worsening effect is called as antagonistic and the effect which is less than synergistic but not antagonistic termed as indifference the Loewe additivity [5]. FICI= 1 (IND can be declared), FICI $\leq$ 0.5 (SYN can be declared) and FICI > 4 (Antagonism) [6]. Some times researcher used Abbott formula [IR= Interaction ratio,

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IR= 0.5-1.5 (Additive can be declared), IR>1.5 (synergism can be declared), IR<1.5 (antagonism can be declared)] [7] and isobolographic analysis to define drug interaction.

Isobolographic Analysis

Isobolographic analysis has previously been applied for the pharmacodynamic study of several classes of non-antimicrobial agents, including antineoplastic, cardiovascular, antiepileptic, analgesic, and anti-inflammatory compounds. Recently, it has been applied in studying pharmacodynamic interactions between azoles and polyenes. It may be a more sensitive method to determine *in-vitro* pharmacodynamic interactions between antifungal and non-antifungal agents. This analysis is based on the Loewe additivity (no-interaction) theory; for details see [8].

While the FICI and its variants have long been employed to depict the characteristics of antimicrobial drug combinations, these approaches have had a number of limitations that have been well described by others [9]. Applying these ideas to *in vivo* and clinical investigations of combination antifungal therapy is especially difficult, and no standards for interpretation of these data have been recommended to date. Analysis and comparison of results across *in vivo* and clinical studies require careful consideration of the nature of pathogen, host, host immune status, study design and study endpoints, for details see [10]. The drugs target on separate cellular pathways may increase the efficacy of "*classic drugs*". The *in-vitro* investigations comprise the bulk of literature on this topic. Therefore, this review mainly summarizes *in-vitro* study of adjunctive combination therapy against IA along with a brief account of clinical data.

ADJUNCTIVE AGENTS: COMBINATION THERAPY

The term "*adjunctive agents*" is taken to include a variety of compounds that are employed in the management of pathological conditions of non fungal infectious etiology but have been shown to exhibit broad-spectrum antifungal activity. Since fungi are eukaryotic cells consequently they share many pathways with human cells and thus increasing the probability of antifungal activity of adjunctive agents. These adjunctive agents include calcineurin inhibitor, Hsp90 inhibtor, chelating compounds, antiarrythmic drugs, antibacterial drugs, immune therapies [Colony stimulating factors (CSF), INF γ monoclonal antibody (Mab)] & other compounds. The potential of these agents for treatment of fungal infections had investigated intermittently alone or in combination with "*classic antifungal drugs*". At this juncture a list of adjunctive agents which show antifungal activity alone and in combination against *Aspergillus* spp. is given in Table **1**.

Calcineurin Inhibitor

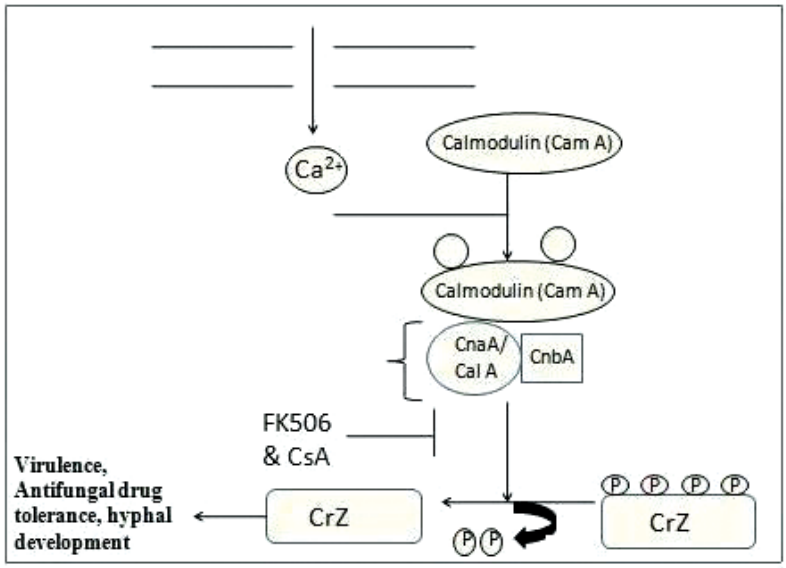
Calcineurin plays an important role in fungal morphogenesis and virulence, as it is involved in regulation of cell wall biosynthesis, translocation of transcription factors, cell cycle progression, hyphae elongation, cation homeostasis and plays an essential role in the regulation of the intracellular Ca^{2+} concentration. It is highly conserved

Table 1. Antifungal Activity of Adjunctive agents against *Aspergillus spp.*

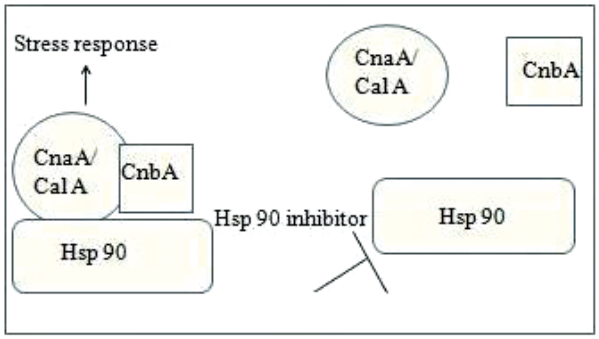
Drugs	MIC (μg ml^{-1})	Drug/Effect in Combination		References
		Drug	Effect	
AMD	16	ITZ	SYN, ADD	[20]
NIF	15	ITZ	SYN, ADD	[20]
Sulfa drugs	64-256	-		[22]
Rifampicin	>1000	AmpB	SYN	[24]
Glycopeptides	-	Echinocandin	SYN	[25]
FK506 (TcS)	1.56	CAS, VCZ	SYN, IND	[15]
CsA	6.25	CAS, VCZ	SYN, IND	[15]
Defrasirox	25	Liposomal AmpB	SYN	[28]
Deferiprone	1.29 ± 0.2 mM	FLZ , AmpB	SYN	[29]
Lactoferrin	105 ± 9 nM	Amphotericin B	SYN	[29]
EDTA	-	LAmpB	SYN (murine model)	[33]
Ibuprofen	>1000	ITZ, VCZ, AmpB	IND	[34]
Gs- CSF	-	POS	SYN	[59]
MAb A9	400	-	(murine model)	[62]
Statin, FLV	2	AmpB	ADD	[7]
Statin ATO	64	AmpB	ADD	[7]

Ca^{2+} - calmodulin activated (Serine/ threonine) protein phosphatase that is important in mediating cell stress responses. It is a heterodimer composed of a catalytic A and regulatory B subunit. The activation of phosphatase function of catalytic subunit requires an association between the two subunits. Following mobilization of internal calcium stores or an influx of Ca^{2+} from outside of the cell, the catalytic subunit bound by Ca^{2+} - calmodulin, freeing the active site from occlusion by a now displaced autoinhibitory domain [11]. The calcineurin signaling pathway is well established in *Saccharomyces cerevisiae* but in *A.fumigatus* is not fully understood (Fig. **1**).

Cyclosporine A (CsA) (Fig. **2B**) and tacrolimus (TcS, FK506) (Fig. **2A**) were originally developed for their antifungal activity but later found to have immunosuppressive activity, the amino acids MeBmt ((4R)-4-[(*E*)-2-butenyl]-4,*N*-dimethyl-L threonine), Abu (2-Aminobutyric acid), Ser, and MeVal are essential for the immunosuppression. These immunosuppressants have revolutionized modern transplantation but their role as potential antifungals is, only beginning to understand. The target of these drugs is calcineurin. The activity of both these calcineurin inhibitors requires cis-trans isomerase immunophilins; CsA binds to cyclophilin A and TcS binds to FKBP [12]. In order to decrease the immunosuppressive activity of these compounds, many investigators produced CsA and TcS analogues that exhibited antifungal activity without the immunosuppressive action. In a model of invasive aspergillosis, survival was significantly prolonged using TcS or sirolimus compared to CsA. Histological examination revealed widely disseminated *Aspergillus* hyphae in the brains of CsA-treated mice, whereas the brains of TcS treated mice showed an almost total absence of hyphae. A previous *in-vitro* disk diffusion study showed that TcS and sirolimus possessed activity against *A. fumigatus* [13]. The *in-vitro* combination study of an antifungal and



A



B

Fig. (1). A, Calcineurin signaling pathway in *A. fumigatus*. Catalytic subunit (CnaA/CalA) bind to regulating subunit (CnbA*) and to calmodulin (CamA*)-Ca^{2+} complex which then dephosphorylates Zinc finger transcription factor. *Components not yet described so arbitrary gene descriptions given to them; B, Role of Hsp90 inhibitor – Left side shows Hsp90 stabilizes the calcineurin helpful for fungal cell to survive under stress, Right side shows compromising of Hsp90 function attenuates calcineurin function.

immunosuppressant used with *Aspergillus* spp. utilized disk diffusion assays and found that 1 µg of caspofugin (CAS) combined with 1 µg of L-685,818 yielded enhanced activity against 8 of 11 clinical *A. fumigatus* isolates [14]. Recently Steinbach *etal*., studied the *in-vitro* interaction between antifungal and immunosuppressant drugs and describe the synergistic and additive effect between the drugs; CAS alone fungistatic but with FK506 showed fungicidal activity [15].

Hsp 90 Inhibitor

Hsp90 is a molecular chaperone that is induced by stress in eukaryotes and regulates the folding, transport of client proteins and influence evolution by releasing previously silent genetic variation in response to environmental change. The molecular chaperon Hsp90 facilitates the emergence and maintenance of fungal drug resistance. The key mediator Hsp90-dependent azole resistance is calcineurin [11], an Hsp90 client protein. Hsp90 binds the catalytic subunit of calcineurin and keeps it poised for activation. Calcineurin activation is required for tolerance of a myriad of environmental stresses, including the membrane stress exerted by azoles [16]. By chaperoning calcineurin, Hsp90 regulates membrane stress responses that are crucial for cells to survive in presence of azoles (Fig. **2**). Hsp90 inhibitors analogues of geldanamycin (GdA) (Fig. **2C**) enhance the efficacy of echinocandin CAS and also enhance the efficacy of voriconazole (VCZ) against *A.fumigatus*. In a wax moth larvae model, combination therapy with GdA + CAS improved survival of the otherwise lethal infection treated with either monotherapy [17]. Hsp90 inhibitors are in currently in phase II clinical trials as anticancer agents and calcineurin inhibitors are widely used deployed as immunosuppressant. Hsp90 and calcineurin are highly conserved regulators of cell signaling in eukaryotes, identifying fungus – specific components of this cellular circuitry with a global impact on drug resistance and virulence would provide novel therapeutic targets.

Antiarrhythmic Drugs

Calcium and its binding protein calmodulin are known to modulate the proliferation, differentiation and metabolism of a variety of cell types. Calcium channel antagonists influence the calmodulin system. The concentration of free intracellular calcium can be increased in eukaryotic cells by opening the voltage-dependent calcium channels (VDCC), allowing extracellular Ca^{2+} to enter the cell. Several chemically distinct classes of organic compounds share the ability to inhibit calcium influx by blocking the calcium channels. These channels are present in yeasts and moulds and therefore are a potential target for new antifungal compounds. Antiarrythmic drugs were reported to cause disruption of calcium homeostasis which has been involved in the antifungal activity. It was hypothesized that the electrophysiological and pharmacokinetic properties of these drugs are closely related to the size and branching of the ester group, lipophilic character of the molecule or substituents. In calcium channel blockers, however, the dominant effect is seen to be of steric factors. The steric roles may be essential in drug-receptor interactions, which seem to involve both hydrophobic, and to a lesser extent, electronic interactions [18].

The antiarrythmic drugs Amiodarone (AMD) (Fig. **2D**) and Nifedipine (NIF) (Fig. **2E**) alone or in combination with Itraconazole (ITZ) were tested against different strains of *Aspergillus fumigatus*. These drugs in combination potentiate the activity of ITZ data shown in Table **1** [19, 20]. Intracellular free calcium ions are thought to be an important second messenger for many neutrophil functions, including phagocytosis, so calcium blockers that have antifungal action may also block the killing activity of human monocytes, thereby limiting the potential use for treating invasive fungal infection.

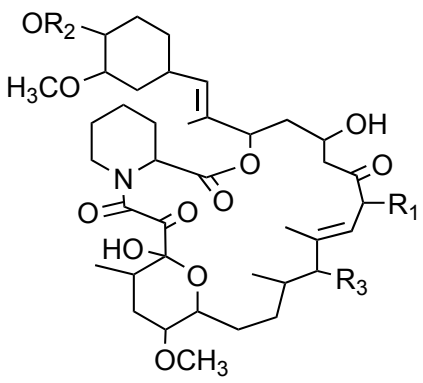
Antibacterial Drugs

Antibacterial drugs such us sulpadrugs, rifampicin (Rif), glycopeptides and ciprofloxacin were studied alone or in combination with polyenes or azoles. Sulpha drugs have been used for antimicrobial chemotherapy since their discovery in 1932; require a basic amino group that should be free to conjugate with sulfamyl group. Introducing an electron withdrawing on the ring increased the antimicrobial activity remarkably [21]. These drugs play their role by interfering the folic acid synthesis. The sulfa drugs sulfamonomethoxine & dimethoxine, sulfadiazine, sulfisoxazole, sulfaphenazole were tested alone against *Aspergillus* spp. showed activity from 64-256µg/ml but there was no data available about their use in combination [22].

Ciprofloxacin has no intrinsic antifungal activity but it may interact with antifungal agents as it inhibits DNA gyrase (topoisomerase II), abundant in fungi. It was first patented in 1983 by Bayer A.G. The link between the carboxylic acid group and the keto group is generally considered necessary for binding of these drugs to DNA gyrase. The conventional fractional inhibitory concentration index analysis was unable to detect interactions between ciprofloxacin and antifungal agents. However, isobolographic analysis revealed significant pharmacodynamic interactions between antifungal agents (AmpB, VCZ, CAS) and ciprofloxacin against *A. fumigatus* strains [8].

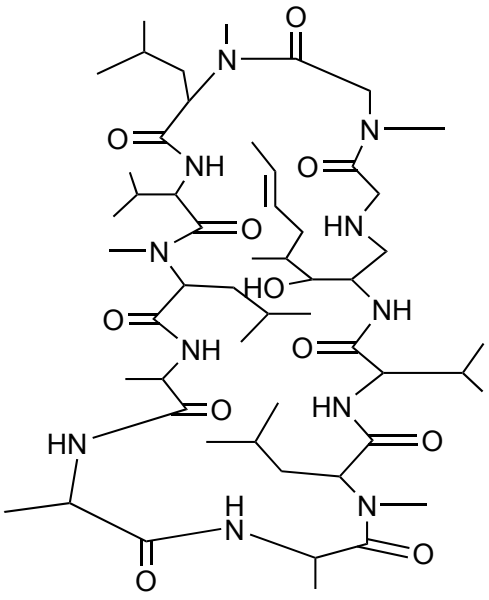
Rifampicin is a macrocyclic antibiotic produced by *Streptomyces mediterranei*. A large number of Rif derivatives have been investigated for antimicrobial activity. The modifications that alter the conformation of the ansa bridge reduce activity. Other structural features of the antibiotic that is particularly critical for activity include the napthol ring with oxygen atom and unsubstituted hydroxyls. Most Rif modifications that retain activity involve substitutions at C3 the napthol ring, which have only modulatory effects on *in-vitro* activity [23]. Single drug does not produce any effect on fungal growth but in combination with AmpB synergistic interaction was observed against yeasts and dimorphic fungi *Aspergillus* spp. *in-vitro* [24].

A novel combination therapy has been disclosed in a patent [25]. The invention describes the administration of an echinocandin in combination with a glycopeptide for invasive mycoses. Glycopeptides are antibacterial drugs, with vancomycin and teicoplanin being the main clinically used agents. The author details reveal surprising fact that the efficacy of an echinocandin can be significantly improved when coadministered with a glycopeptide having a substituent comprising at least about 8 carbon atoms

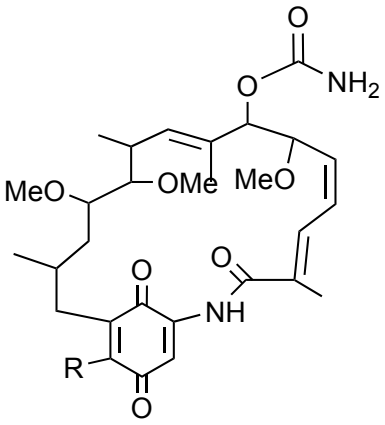


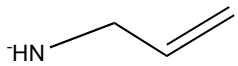
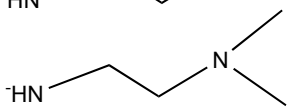
Compound	R_1	R_3
Tacrolimus	H_2C -	-H
L_ 685818	C_2H_5	-OH

Tacrolimus & its analog (A)

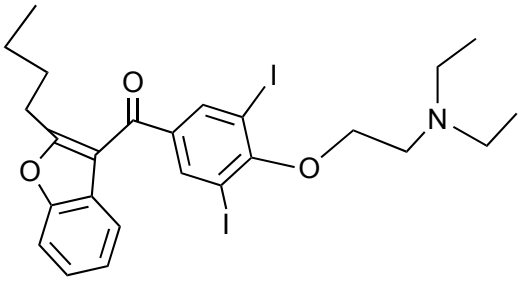


Cyclosporine A (B)

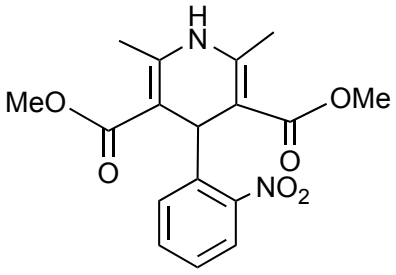


Compound	R
GdA	OCH_3
17-AAG	
17-DMAG	

Geldanamycin A & its analog (C)



Amiodarone (D)



Nifedipine (E)

Fig. (2). A, **B**, Structure of Calcineurin inhibitor; **C**, Hsp90 inhibitor; **D**, **E**, Antiarrythmic Drugs.

(FIC against Aspergillus spp. ranging from 0.16 to 0.31μg/ml). One has to cautious about the side effects of glycopeptides such as the emergence of vancomycin-resistant strains of enterococci (VRE) while dealing with them.

OTHER COMPOUNDS

Statin

They are inhibitors of HMG-CoA reductase to lower the cholesterol level. The *in-vitro* interactions between polyene antifungal drugs and different statins were evaluated using a standard chequerboard broth microdilution method. The statin PRA (Provastatin) (Fig. **3A**), FLV (fluvastatin) (Fig. **3B**) and ATO (Atorvastatin) (Fig. **3C**) were combined with Amp B against *Aspergillus* spp. Most of the detected interactions were additive. These interactions were observed by using Abbott formula [7], data shown in Table **1**.

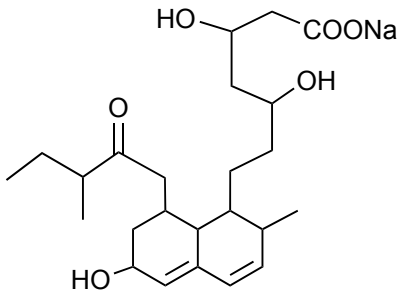
Chelating Compounds

Chelating agents have been shown to have antifungal activity *in-vitro* [26]; they are involved in chelation of cations like iron - required for growth of fungus and calcium - activation of calcineurin which is required for morphogenesis or fungus virulence and thereby potentiate the activity of the ampB-based antifungal regimen. Laboratory studies also demonstrate that iron acquisition is essential for the growth and virulence of *Aspergillus*. Most recently it has been reported that an increased bone marrow iron level was an independent risk factor for developing IPA (Invasive Pulmonary Aspergillosis) in high-risk patients. Therefore, chelating host iron with an appropriate agent might improve the outcome of IPA [27].

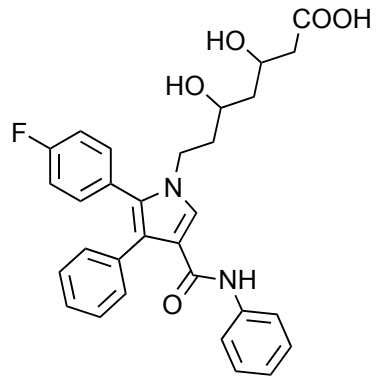
Deferasirox (Fig. **3D**) is the first orally available iron chelator approved by the FDA, with an indication for the treatment of transfusion related iron overload. It has been found that deferasirox is highly active against *Aspergillus*, has a significant efficacy alone as well as in combination therapy with lipid polyenes for the treatment of IPA [28]. Antifungal effects of other iron chelators (lactoferrin, deferoxamine and deferiprone) were tested alone and in combination with antifungal drugs against *Aspergillus fumigatus* B5233 conidia. Lactoferrin and deferiprone inhibited whereas deferoxamine enhanced fungal growth. Antifungal synergy against conidia was observed for combinations of KTZ with deferiprone, lactoferrin with AmpB, and fluconazole (FLZ) with deferiprone [29]. The other chelator, such as EDTA is a well known chelator which has shown to have antifungal activity *in-vitro* [26]. As much literature has written on its synergistic or potentiating action with common preservatives, antibiotics and cationic surfactants [30-32]. Recently EDTA has found to act as an adjunct antifungal agent for IPA in a rodent model with combination of ABLC [33].

Ibuprofen

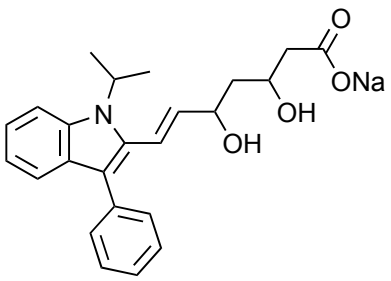
Ibuprofen (IBU) a propionic acid derivative is a non-steroidal anti-inflammatory drug widely used in clinical practice. The analgesic, anti-inflammatory, and antipyretic



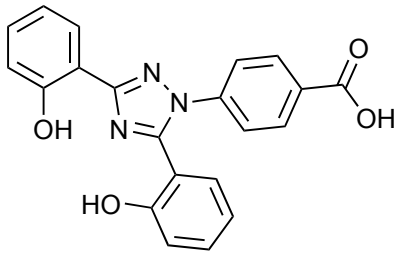
Provastatin (A)



Fluvastatin (B)



Atorvastatin (C)



Deferasirox (D)

Fig. (3). Structure of Other Compounds used in Combination **A**, **B**, **C** Stain; **D**, Chelating Compound (Deferasirox).

action of IBU is believed to be the result of its ability to block the cycle-oxygenase (COX) enzyme system, thus inhibiting the synthesis of prostaglandins from the precursor, arachidonic acid. It exhibited weak fungal activity alone and in combination with ITZ and VCZ [34].

DRUG INTERACTION

Metabolic drug interactions between drugs represent a major concern for the pharmaceutical industry, for regulatory agencies and clinically for health care professionals and their patients. Therefore, drug interactions between antifungal and adjunctive agents are of interest to be taken in to account.

Azole drugs are well known for their numerous drug-drug interactions which are revealed by several studies [35]. They serve as inhibitors of several CYP 450 isoenzymes (Fig. **4**) (plays a dominant role in the biotransformation of a vast number of structurally diverse drugs) to a varied extent [36], which may contribute to significant drug interactions (Table **2**). The nuclear receptor, pregnane X receptor (PXR; NR1I2) is key transcriptional regulator of drug-metabolizing enzymes, including CYP3A4. In its active form, PXR is in complex with heterodimerization partner retinoid X receptor alpha (RXRα; NR2B1) [42] and with several coactivators, such as steroid receptor co-activator 1 (SRC-1) and hepatocyte nuclear factor 4 alpha (HNF4α) [43]. It has been found that KTZ disrupted the interaction between PXR and SRC-1 [44] & the interaction of PXR with HNF4α might be involved in KTZ-mediated inhibition of CYP3A4 gene expression [45]. The additive and antagonistic interactions of azoles with rifampicin on PXR transcriptional activity and CYP3A4 expression was reported [46]. It was observed that KTZ suppressed rifampicin-mediated CYP3A4 transactivation and SRC-1 recruitment to PXR, which is in accordance with data from other authors [44]. In addition, KTZ diminished interaction of rifampicin with PXR ligand binding domain.

The role of Glucocorticoid receptor (GR) in the regulation of drug-metabolizing cytochromes P450 is very complex, comprising several mechanisms. Apart from regulating the regulatory proteins (PXR, CAR, RXRs etc.), GR is involved in direct (cis) and indirect (trans) transcriptional regulation of P450s [47]. For instance, functional GR response elements were identified in promoters of human CYP2C8 [48], CYP2C19 [49] genes. Trans-regulation of human CYP2A6 [50] and CYP3A4 [51] by GR, was demonstrated, involving HNF4α in the process. Therefore, antagonism of GR by KTZ and miconazole results in down-regulation of cytochromes P450.

It has already been shown that tacrolimus bioavailability almost doubles when co administrated with KTZ [52]. Antifungal agents are known to inhibit cytochrome P450 3A4/5 (CYP3A4/5) enzymes. CYP3A4/5 is also involved in the metabolism of cyclosporine, tacrolimus, and sirolimus. Preliminary observations indicate that voriconazole at a dose of 200 mg twice a day increases the trough concentrations of cyclosporine in blood of transplant patients. It has been found that voriconazole inhibit the metabolism of tacrolimus by 50% at 10.4 ±4.3 μg/ml [53].

The antiarrythmic drugs are (AMD and verapamil) inhibitors and substrate for *CYP3A4*, CYP2C9 (involves in metabolism of drugs) and P-glycoprotein (involves in excretion of drugs) [54]. Therefore theoretically it might seems that combination of non- antifungal drugs with "classic" antifungal agents might improve outcome but very little is documented about drug–drug interactions, only

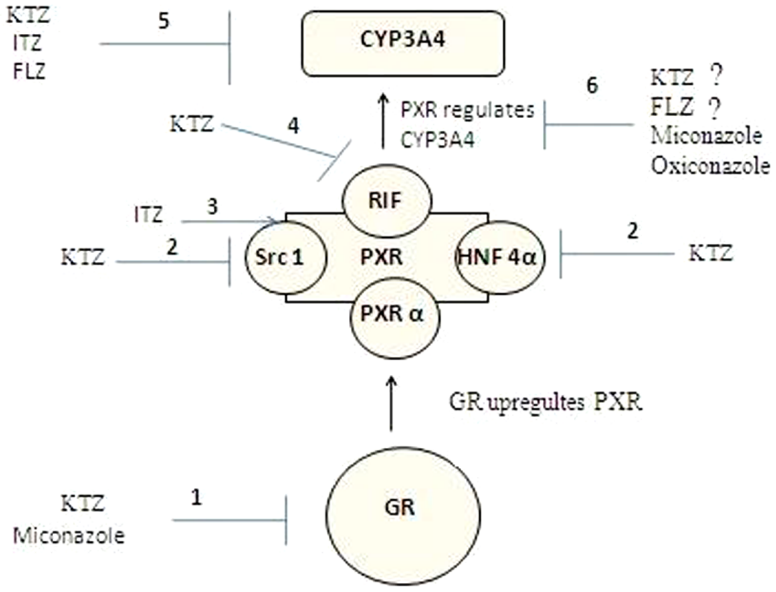


Fig. (4). Role of Azole in drug-drug interaction through inhibition of metabolizing enzyme & receptor, (1)-KTZ & Miconazole are inhibitors of GR; (2) – KTZ disrupts interaction between HNFα & PXR, Srcl & PXR; (3) – ITZ promotes interaction between Src1 & PXR; (4) – KTZ binds to PXr in ligands binding domain; (5) – KTZ, ITZ FLZ inhibit catalytic activity of CYP3A4; (6) – KTZ, FLZ, Miconazole, Oxiconazole inhibit/potentates PXR – mediated interaction of CYP3A4.

Table 2. Selected Drug-Drug Interactions with Azole agents

Drugs	Fluconazole (FLZ)	Itraconazole (ITZ)	Voriconazole (VCZ)	Posaconazole	References
CsA	Low-dose FLZ has minimal effect; higher dosing increases CSA	CSA AUC levels increased 50-80%. Monitor and reduce CSA dose by 50%.	CSA levels increased 70%. Monitor and reduce CSA dose by 50%	CSA level increased in cardiac transplant recipients.Consider 30% dose reduction of CSA	[37]
Sirolimus	Large increase in sirolimus levels seen by day 3 after initiation of FLZ; consider 50-75% sirolimus	Single dose sirolimus in patients on KTZ increased .	Single VCZ dose leads to 7-fold increase in sirolimus	Not documented. Increase in sirolimus levels likely.	[37, 38]
Tacrolimus (FK506)	FLZ doses increase FK506 Consider 50% dose reduction of tacrolimus.	FK506 level increased with ITZ. FK506 increased 88% when both drugs IV; reduce tacrolimus 50% .	FK506 AUC triples; reduce tacrolimus dose to 1/3 and monitor levels	Single dose study: 358% increase AUC; consider dose reduction and monitor levels	[39, 40]
NIF	Not documented. Increased nifedipine levels theoretically possible. Monitor for hypotension	Increased nifedipine levels likely. Monitor for hypotension	Not documented. Increased nifedipine levels likely. Monitor for hypotension	Not documented	[37]
Statin	Conc. Increase	Conc. Increase	Conc Increase	Conc. Increase	[41]

interactions of azoles with non-antifungal are very much talked about as these are one of the most frequently used drugs in human pharmacotherapy.

ADJUNCTIVE COMBINATION IMMUNE THERAPIES

Antifungal therapy is often ineffective in setting of immune suppression but intends to enhance immune function. Hence, immunotherapy is a rational approach in treating fungal infections. The immunomodulators (CSFs, INF γ) and MAb (adjunctive agents) can be used alone and in combination with "*Classic antifungal drugs*".

Colony-Stimulating Factors

CSFs regulate leukocyte maturation from bone marrow progenitor cells. Three types of recombinant human CSFs are available: granulocyte CSF (G-CSF), granulocyte macrophage CSF (GM-CSF), and macrophage CSF (M-CSF); for reviews, see [55]. Immunocompromised patients receive therapy with immunomodulator granulocyte colony stimulating factor (G-CSF) to boost host defence against inhaled *Aspergillus* spp. It has been suggested that G-CSF not only augments neutrophil activity but also improves the effects of antifungal drugs [56]. So far aerosolized GM-CSF has shown to be safe and well tolerated by patients in studies of metastatic sarcomas of the lungs and in pulmonary alveolar proteinosis. The intranasal granulocyte-macrophage colony-stimulating factor given to immunosuppressed mice infected with pulmonary aspergillosis resulted in reduction of fungal growth. It can be a novel therapeutic approach for the prevention pulmonary fungal infections [57].

Triazoles, including POS (Posaconazole), inhibit lanosterol 14-α demethylase. The net result is a depletion of ergosterol and the accumulation of methylated precursors in the fungal membrane. These changes in membrane composition may result in increased sensitivity to oxygen-dependent immune mechanisms such as neutrophil attack. The combination of POS and G-CSF offers the possibility of direct inhibition of fungal growth, triazole-induced susceptibility to neutrophil-mediated killing, and enhancement of innate immunity by G-CSF. *In vivo* studies using G-CSF–triazole combination therapy have reported either a beneficial response or no significant difference [58] in the survival of infected mice. Recently the combination of G-CSF with POS has been studied and it has been found that it does not substantially affect the antifungal efficacy of POS in a murine model of invasive aspergillosis, with differences observed in ranges similar to those of previous studies that reported a lack of effect of G-CSF on triazole efficacy. They also studied the route of infection may play major role in susceptibility and response to therapy, an intranasal route of infection with *A. fumigatus* ND208. An interesting difference was found in the clearance of *A. fumigatus* ND208 infection after intranasal or inhalation administration. This finding may suggest possible differences in mechanisms and efficiency of pathogen clearance following different routes of infection. It is conceivable that the infectivity and dissemination of *Aspergillus* may differ in the lungs depending on whether the infective dose is administered in solution or by aerosol, and as a result, the host response may also be affected. Generally aerosolized infections result in diffuse bronchopneumonia with even distribution and replication primarily in lung tissue, whereas intranasal administration often results in upper respiratory tract infection. Thus, diffuse *Aspergillus* infection resulting from aerosol administration may contribute to the increased mortality and lung burden observed [59].

IFN-γ

IFN-γ is a potent activator of macrophage function that can enhance the antifungal activity of murine macrophages against a number of fungal pathogens both *in-vitro* and *in vivo*. The efficacy of IFN-γ against human fungal infections has not been extensively studied. However, there is sketchy evidence suggesting that IFN-γ can be useful adjunctive therapy for the treatment of certain unusual fungal infections. Synergy of IFN-γ with antifungal agents against *A. fumigatus* and *Candida spp*. has been demonstrated *in-vitro* [60].

Monoclonal Antibody

The pathogenicity and virulence biological functions reside in the fungal cell wall mediates the host-fungus interplay [61]. This includes triggering and modulation of host immune responses. The identification and characterization of cell wall immunodominant proteins eliciting potent immune responses during aspergillosis could have important repercussions for developing novel diagnostic and therapeutic techniques for aspergillosis. Due to these reasons much effort has been focused on the discovery of useful inhibitors of cell wall glucan, chitin, and mannoprotein biosynthesis. In the absence of a wide-spectrum, safe and potent antifungal agent, a new strategy for antifungal therapy is directed towards the development of MAbs. The efficacy of any particular MAb depends on several variables such as the characteristics of the targeted antigen, its function and its cell surface density as well as characteristics of the MAb, including specificity, avidity and isotype. MAbs have served as useful research tools and has dramatically improved the specificity of immune procedures. Some of applications of MAbs are immunochemical characterization and purification of bacterial, fungal, or viral antigens; localization of viral and fungal glycoproteins and development of antibody and antigen detection assays. MAb A9, which is IgG1 generated against cell wall glycoprotein of *A. fumigatus*, showed promising results *in-vitro* as well as *in vivo*. MAb A9 uniformly opsonized the cell surface and was found to be protective in an experimental murine model of invasive aspergillosis [62].

This is a brief of adjunctive therapies which have studied alone and in combination with standard antifungal drugs against *A.fumigatus* to maximize the efficacy of antifungal therapy. Apart from above mentioned therapies there is a new approach strived to fight against fungal infection i.e Radioimmunotherapy (RIT).

RADIOIMMUNOTHERAPY

The utilization of radiolabelled monoclonal antibodies for treatment of infectious disease is reported [63]. RIT is well established as a treatment modality for cancer but there is limited information about its use against infectious disease. RIT depends on antibody-antigen interactions and utilizes antibodies radiolabelled with therapeutic radioisotopes to deliver lethal doses of radiation to target cells. The radiolabeled peptides are promising nuclear imaging agents because of their pharmacokinetic properties, rapid binding and relatively low immunogenicity in early diagnosis of fungal infection in IPA. The ^{111}In-labeled c (CGGRLGPFC)-NH2 can selectively accumulate in infected lungs; therefore it may facilitate diagnostic imaging of *A.fumigatus* infection. It could be labeled with ^{111}In through the DTPA (Diethylenetriamene pentaacetate) chelator with high radiolabeling efficiency and high stability. Using an established murine model of IPA [64] found that ^{111}In-DTPA-c (CGGRLGPFC)-NH2 was selectively localized to lungs infected with *A.fumigatus*. The uptake of the radiotracer in the lungs of the infected mice was more than twice that in the lungs of the healthy mice. c (CGGRLGPFC)-NH2 was found to reliably bind to surface of *A. fumigatus* hyphae, binding may not have been specific to the *A. fumigatus*, since it also binds to hyphae of other *Aspergillu*s species.

BRIEF REVIEW OF CLINICAL DATA: COMBINATION THERAPY

The clinical data till date with adjunctive agent is not adequate. The range of data from synergy to antagonism actually parallel the wide range of unproven treatment practices used by clinicians today searching for the best care for their patients. Only a few studies are available like Denning and Stevens previously reviewed a total of 2,121 cases in 497 articles concerning clinical aspects of IA from 1966 to 1990 and revealed 89 clinical cases of combination therapy in 54 articles: AmpB + /rifampin (26 cases) and AmpB +/5-FC (63 cases). The largest analysis of combination therapy for IA show three combination regimens comprised the majority of reported clinical experience, as many (49%) involved AmpB+/5-FC (Fluorocytosine), while AmpB+/ITZ (17%) and AmpB + Rif (11%) were less common [65, 66]. However, after including the lipid formulations of AmpB, i.e. AmpB Lipid Complex (ABLC), AmpB Colloidal Dispersion (ABCD), or Liposomal AmpB (LAmpB), the frequency of those three combinations increased from 77% to 89% of the total number of combinations analyzed. In this study also only one adjunctive agent is included i.e Rif. Same way clinical experience with use of the CSFs to treat fungal infections is limited, although there is growing anecdotal evidence that they may alter the course of established fungal infection, when used as adjuncts to antifungal therapy. A number of reports suggest that G-CSF may be useful as adjunctive therapy for certain fungal infections in combination with AmpB; these include reports of 5 children with aspergillosis [67], patients with fungemia in the setting of hematologic malignancy [68]. A study of 29 patients with deep-seated fungal infections following chemotherapy or bone marrow transplantation reported that combined therapy with Amp B and G-CSF was associated with an improved response rate and greater cost-effectiveness [69]. Recently adjuvant therapy with INF- γ was added to the antifungal treatment in 3 neutropenic patients of pulmonary aspergillosis (one HIV and 2 Non HIV patients), there has been increase in the production of type –I cytokine & IL-4 which help in the clearance of *Aspergillus* infection [70].

The current Infectious Disease Society of America guidelines for treating IA [71] State that combination therapy does have a potential role in therapy but the data is obviously unclear.

CONCLUSION AND FUTURE DIRECTIONS

Adjunctive Combination therapy is an attractive concept for treating invasive mycoses but optimal regimens remains unclear. There is much *in-vitro* data present but as we move forward using of this systemic investigation there is a need for more animal studies that take pharmacodynamics into account. We must consider the potential risks, benefits of these approaches and design these studies with utmost care. Future attention towards issues like dose response, dose selection, and drug- drug interaction is mandatory.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

ACKNOWLEDGEMENTS

The authors wish to thank Council of Scientific and Industrial Research (CSIR), University Grant commission (UGC), Haryana State Council for Science and Technology (HSCST), India for the necessary support.

ABBERIVATIONS

FIC	=	Fractional inhibitory concentration
FICI	=	Fractional inhibitory concentration index
MIC	=	Minimum Inhibitory Concentration
AMD	=	Amiodarone
NIF	=	Nifedipine
AmpB	=	Amphotericin B
Rif	=	Rifampicin
LAmpB	=	Liposomal Amphotericin B
ABLC	=	Amphotericin B lipid Complex
ITZ	=	Itraconazole
VCZ	=	Voriconazole
KTZ	=	Ketoconazole
FLZ	=	Fluconazole
CAS	=	Caspofungin
CsA	=	CyclosporineA
TcS	=	Tacrolimus
GdA	=	Geldanamycin A
IA	=	Invasive Aspergillosis
IPA	=	Invasive Pulmonary Aspergillosis
MAb	=	Monoclonal antibody
GS-CSF	=	Granulocyte Colony Stimulating Factor
SYN	=	Synergy
ADD	=	Additive
IND	=	Indifference
RIT	=	Radioimmunotherapy

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Received: January 22, 2012 Revised: May 05, 2012 Accepted: May 10, 2012