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Drug resistant fungal infections-An emerging threat

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Abstract

Drug resistance has become an important problem leading to significant negative social, psychological, and occupational health effects and quality of life. Clinical failure and relapses have been observed in patients treated with antifungal drugs. Now a days, Antifungal drug resistance is becoming an emerging threat in management of fungal infections especially in immunocompromised patients. Early recognition and treatment is essential to reduce morbidity and possibility of transmission. The increased use, inappropriate prescribing and over the counter sale of antifungal agents has also added in the development of resistance to these drugs. The level of resistance to antifungal agents is still relatively low, but there is a possibility of antifungal resistance becoming a crucial determinant of outcome following antifungal therapy in future. In addition to discussing the molecular mechanisms of antifungal resistance, this article elaborates on the current concept of clinical resistance, which is critical to the understanding of treatment failure in patients with fungal infections.

Key words: Antifungal drugs, Resistance, Invasive fungal infections Amphotericin, Polyene, Azole, Echinocandin, Flucytosine, Terbinafine, Griseofulvin

Introduction

Fungal infections are a worldwide global health problem, affecting millions of patients per year. Of these, approximately 1.5 million are disseminated or invasive fungal infections (IFIs), requiring advanced treatment and hospitalization.[1] Unfortunately, this high number of infections is associated with high mortality rates, with some fungal infections having mortality rates nearing 90%–95%.[2]

The most frequent fungal pathogens are Candida, Aspergillus, Pneumocystis, and Cryptococcus spp. It is estimated that these fungal species cause at least 1.4 million deaths worldwide per year.[3] Compared to other microbial pathogens causing bloodstream infections, Candida spp. are ranked fourth among the most common agents of bloodstream infections, after other common bacterial pathogens. Aspergillus infections are the most common microbial infections in hematopoietic stem cell transplant (HSCT) recipients. About 30–50% of invasive aspergillosis patients still die, and the mortality from candidemia also remains high at ~50%.[4]

Due to the high global health burden associated with fungal disease, the treatment of these infections needs to be potent and effective. Indeed, many of the currently available classes of antifungal drugs are highly effective in the appropriate contexts. However, these drugs, as with any therapy, have limitations and caveats.[5]

Moreover, the problem of antifungal resistance is on the rise: both that which has evolved in formerly sensitive species, as well as the prevalence of intrinsically-resistant species of fungi. To date, resistance exists to all of the currently available classes of antifungal agent.[6] Candida species have a high prevalence of azole resistance, largely attributed to the cytostatic nature of these drugs.7 Similarly, Aspergillus and Cryptococcus strains have recently also demonstrated azole resistance.[8] Only a few years ago, echinocandins were considered effective therapy for most clinically-relevant Candida isolates. However, with increased use of these antifungal agents, echinocandin resistance in Candida species has also become more prevalent.[9] Additionally, the intrinsically drug-resistant fungi, such as Scedosporium species, continue to cause a background of infections in highly immunosuppressed patients, especially those who are heavily treated with antifungals. These infections are often associated with poor patient outcomes. Due to these limitations, there is an urgent need for new antifungal agents.[10]

Antifungal drugs and their mechanism of action

There are only four major classes of antifungal drugs available to treat invasive fungal infections. They include

polyenes, pyrimidine analogs, echinocandins, and triazoles. A fifth class (allylamines) is also existing; however, compounds of this class (for example, terbinafine) are used only for treating superficial dermatophytic infections.[11] Polyenes, such as amphotericin B (AmB), have the ability to bind ergosterol and act as a sterol "sponge," thus destabilizing membrane functions. Ergosterol is a major sterol of fungal membranes and is required for maintaining cell membrane integrity. AmB may exert intrinsic toxic effects in humans; however, this negative effect can be avoided by using liposome formulations. Pyrimidine analogs, such as 5-fluorocytosine (5-FC), are metabolized by fungal cells into fluorinated pyrimidines, which destabilize nucleic acids (RNA, DNA) and therefore result in growth arrest. 5-FC is used mainly for the treatment of Cryptococcus spp. meningitis and in combination with AmB.[12] Echinocandins block the catalytic subunit of the β-1,3 glucan synthase and thus inhibit cell wall biosynthesis. In medical practice, triazoles are still the most used antifungals. These compounds target a specific step in the biosynthesis of ergosterol that catalyzes lanosterol 14α-demethylation. Fluconazole is the major triazole in clinical settings, probably due to its high oral availability and tolerability by patients.[13]

Table 1: Antifungal agents against dermatophytes[14]

Chemical group	Site action	Target
Allylamines (terbinafine, Naftifine)	Ergosterol Biosynthesis	Sqyalene epoxidase
Azoles	Ergosterol Biosynthesis	
Imidazoles Bifonazole clafrimazole, Econazole, ketoconazole		Cytochrome 450 14α-Lanosterol Demethylase
Miconazole	Ergosterol Biosynthesis	
Triazoles Fluconazole, Itraconazole, Terconazole	Ergosterol Biosynthesis	Sterol reductase and isomerase
Morpholines (Amorolfine)	Fungal mitotic Apparatus	Membrane barrier function
Polyenes (Amphotericin B, Bystatin)		Squalene epoxidase
Thiocarbamate (Tolnaftate)		
Griseofulvin		Sliding of microtubules

Ideal antifungal agent[14]

At present, the antifungal therapy of dermatophytosis has its own limitations. As dermatologists, we wish all the antifungal drugs to have certain attributes which make them ideal [Table 2]. The search for ideal antifungal agent which can rescue both the dermatologists and patients from the menace of dermatophytosis continues. Meanwhile, we need to critically review and use the antifungal agents rationally.

Table 2: Characteristics of ideal antifungal agent[14]

Highly effective against all dermatophyte
Strong anti-inflammatory action
Rapid action/ response
Good penetration
Good reservoir effect
Minimal systemic absorption
No side effects
Short duration of therapy
Risk of development of resistance
Cost-effectiveness
Safe use in pregnancy and lactation
Safe use in renal and hepatic failure

Mechanism of antifungal resistance

Martinez-Rossi described the antifungal resistance mechanisms in dermatophytes. The main biochemical and molecular mechanisms that contribute to antifungal resistance include reduced uptake of the drug, an active transport out of the cell or modified drug metabolic degradation of the cell, changes in the interaction of the drug to the target site or other enzymes involved in the same enzymatic process by point mutations, overexpression of the target molecule, overproduction or mutation of the target enzyme, amplification and gene conversion (recombination), and increased cellular efflux.[15]

Various mechanisms by which microbial cells might develop resistance include i) The target enzyme is overproduced, so that the drug does not inhibit the biochemical reaction completely, ii)The drug target is altered so that the drug cannot bind to the target, iii) The drug is pumped out by an efflux pump, iv)The entry of the drug is prevented at the cell membrane/ cell wall level, v) The cell has a bypass pathway that compensates for the loss-of-function inhibition due to the drug activity, vi) Some fungal enzymes that convert an inactive drug to its active form are inhibited,vii) The cell secretes some enzymes to the extracellular medium, which degrade the drug.[16]

Recently, a team of researchers using detailed genetic, biochemical, and molecular approaches, identified a mechanism controlling multidrug resistance in fungi. They found that yeast induce multidrug resistance via a molecular switch similar to one that removes drugs and other foreign substances from human cells. When the yeast protein Pdr1p binds to anti-fungal drugs or other chemicals, it switches on molecular pumps that remove the drugs from the cell. The research team also showed

that this chemical switch also controls drug resistance in an important human pathogenic fungus, Candida glabrata.[17]

Heat shock protein 90 also takes part in pathogenesis of resistance to antifungals. The Hsp90 chaperone protein provides one mechanism to link temperature with the signalling cascades that regulate morphogenesis, fungal development and virulence. Targeting the molecular chaperone Hsp90 or its downstream effector, the protein phosphatase calcineurin, abrogates resistance to the most widely deployed antifungals, the azoles, which inhibit ergosterol biosynthesis.[18]

Another emerging source of antifungal resistance is the occurrence of a biofilm, the extracellular matrices produced by microbes themselves which serve to help organisms attach to living or non-viable surfaces . It is estimated that about 65% of all human microbial infections involve biofilms and the majority of invasive diseases produced by C. albicans are associated with biofilm growth. It has been demonstrated that drug efflux pumps play a role in the drug resistance of early biofilms. It has been hypothesized that a change in membrane sterol composition during biofilm formation might explain resistance to amphotericin B and the azoles.[19]

Prevention of antifungal drug resistance & future of antifungal therapy

The primary factor driving the emergence of antifungal resistance appears to be resulting from the increased use and inappropriate prescribing of systemic antifungal agents. There is no clear evidence as to what dosing strategy should be used during treatment and prophylaxis to best avoid resistance.20 Ghannoum and Rice[16] suggested measures to avoid and suppress the emergence of antifungal resistance which include (i) prudent use of antifungals, (ii) appropriate dosing with special emphasis on avoiding treatment with low antifungal dosage, (iii) therapy with combinations of existing agents, (iv) treatment with the appropriate antifungal (in cases where the etiological agent is known), and (v) use of surveillance studies to determine the true frequency of antifungal resistance.

Use of combinations of antifungal drugs or use of adjunctive immunostimulatory therapy may be more effective in preventing development of resistance. A variety of immunosuppressive compounds, including cyclosporin and D-octapeptides, have been tested and found to counteract antifungal resistance due to efflux pumps. Cernicka, et al. screened a synthetic compound library and identified a chemical that increased the sensitivity of a drug-resistant strain of S. cerevisiae to fluconazole. The compound also increased sensitivity of the pathogenic yeasts Candida albicans and Candida glabrata that expressed efflux pumps.[21]

A new way to fight drug-resistant fungal infections targeting heat shock protein 90 has been suggested. Hsp90 acted as a kind of thermostat for C. albicans and shutting down the protein's temperature-sensitivity can shut down the spread of infection. It has been suggested that interfering with Hsp90 function provides a powerful and much-needed strategy to render existing antifungal drugs more effective in the treatment of life-threatening fungal infections.[22]

Photodynamic therapy has been suggested as an alternative treatment for therapy resistant patients, however, the data on this are still limited and in some cases, the aggravation rates are higher than with other methods. An alternative non-invasive treatment protocol utilizing combinations of VIS-NIR laser beams in association with broadband beams of UV, B and R-LEDs, without usage of photosensitizers, with minimal side effects, for therapy resistant patients suffering from Tinea Pedis, Pityriasis Versicolor, or Mycetoma has been demonstrated.[23]

The cellular targets for antifungal action are limited because both human and fungi are eukaryotic organisms.[24] Hence, the strategy to prevent or control of drug resistance is critical [Table 3].[14] Considering the mechanisms of drug resistance, new drug targets have been identified [Table 4].[24] The new drug should act efficiently against wide range of fungal with no or minimal toxicities to host. The cellular target should be essential and conserved in variety of fungi and more importantly its counterpart should not be present in the host.[25]

Table 3: Prevntion or control of antifungal resistance[14]

Judicious use of antifungal agents
Avoiding treatment with low dose of antifungal agents
Combination therapy using drugs with
deferent mechanisms of action
Surveillance studies to identify true nature of antifungal resistance

Table 4: Putative targets for new antifungal agents[24]

Genes and proteins: Involved in infection of host tissues **Virulence factors:** Keratinases, clastases, DNase, proteases, Lipases, Mucolytic enzymes **Sulfite transporters:** Involved in proteolytic digestion of hard keratin Heat shock protein 90 **ATP-Binging cassette transporters:** Invoved in drug efflux
ATP: Adenosine triposphate

Considering the characteristics of ideal antifungal agent, development of novel drug delivery system appears to be the suitable option for management of dermatophytoses [Table 5].[26] They ensure prolonged pharmacological effect by achieving high local concentration of drug in epidermis and dermis through controlled release of drug from the formulation.[26]

Table 5: Novel delivery system for topical antifungal therapy[26]

Micelles
Solid lipid nanoparticles
Nonstructured lipid carriers
Microemulsions
Vesicular delivery systems
Liposomes
Niosomes
Ethosomes
Transfersomes
Penetration-enhancer vesicles

Conclusion

Antifungal drug resistance is becoming a major concern during treatment of patients with fungal infections. A better understanding of the mechanisms and clinical impact of antifungal resistance is essential to the prompt and efficient treatment of drug resistant fungal infections & to improving the outcome of such infections. The modification in pharmacotherapy is not the only solution for current problems associated with fungal infections. The clinical response also depends on the choice of antifungal agent, topical/systemic/both, type, site, and extent of infection, comorbidities, and immune status of the host. We need to focus more on developing an effective strategy to literate the population regarding predisposing factors, adverse effects of over the counter drugs, need of expert consultation, and importance of following expert's advice on management of the disease. It is also essential to literate general physicians about ill effects of steroid combinations and adequate dosage duration of appropriate antifungal drug. Simultaneously, the antifungal agents should be used judiciously to avoid antifungal resistance.

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