



Recent trends and advances in fungal drug delivery

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ABSTRACT

Topical therapy is an attractive choice for the treatment of the cutaneous infections due to its advantages such as targeting of drugs to the site of infection and reduction of the risk of systemic side effects. Optimal selection and dosing of antifungal agents are important, as these infections are often refractory to available therapy. Agents administered for invasive infections are amphotericin B, flucytosine, and azole antifungals. Several drugs are under investigation, such as posiconazole, voriconazole, and the echinocandins, and preliminary pharmacodynamic data likely will help shape dosing regimens. The efficiency of treatment depends on the penetration of drugs through the target layers of the skin at the effective concentrations. However, stratum corneum, the outermost layer of the skin, is an effective barrier for penetration of drugs into deeper layers of the skin. The physico-chemical characteristics of drug molecules and the types of the formulations are effective factors in topical drug delivery. The review article focuses on the new alternative formulation approaches to improve skin penetration of antifungal drugs.

Keywords: Anti fungal agents, topical drugs, *stratum corneum*, amphotericin

INTRODUCTION

Drug delivery is the method or process of administering a pharmaceutical compound to achieve a therapeutic effect in humans or animals. The study of fungus is referred to as mycology. Fungus, in plural called as fungi. Fungi are saprophytic microorganisms which have evolved mechanisms to survive in the mammalian hosts (1). Fungal infections are amongst the most commonly encountered diseases affecting the skin. Anti-fungal drugs are used for superficial and deep (systemic) fungal infection. A disquieting trend after 1950s has been the emergence of more sinister type of fungal infections which are to a large extent, iatrogenic. The term iatrogenic denotes adverse responses of the patient to medical or surgical treatment. These fungal infections are associated with the use of broad spectrum antibiotics, corticosteroids, cytotoxic drug, indwelling catheters and implants and emergence of AIDS. As a result of breakdown of host immune system, saprophytic fungi easily invade living tissues.

Treatment approaches include both topical and oral antifungal agents. In topical administration, the entering of drugs to systemic circulation is prevented or minimized. Thus, the systemic adverse effects of drugs are avoided (2). Besides, topical preparations have better patient compliance due to their non-invasiveness and, they can be self-administered (3, 4). Advances in the field of formulation may soon render outdated conventional products such as creams, ointments and gels. Several carrier systems loaded with antifungal drugs have demonstrated promising results in the treatment of skin fungal infections. Examples of these newer carriers include micelles, lipidic systems such as solid lipid nanoparticles and nanostructured lipid carriers, microemulsions and vesicular systems such as liposomes, niosomes, transferosomes, ethosomes, and penetration enhancer vesicles (5).

Topical agents that are conventionally used for the treatment of skin fungal infections are usually formulated as creams, lotions or gels (6). They either exhibit fungicidal or fungistatic actions depending on the agent being delivered. Since the side effects of fungal agents applied topically are less than their oral counterparts, they are the preferred agents. Another advantage of topical formulation is that it avoids drug-drug interactions, which are more common in case of oral administration (7-9).

In order to achieve a topical effect for an antifungal drug, the release rate of lipophilic drug should be controlled by the formulation in order to achieve high local therapeutic concentration and to provide prolonged pharmacological effect (10). Another important consideration is the molecular weight of the drug (11). Many topical antifungal have been available since the antiseptic era. Two important antibiotics are amphotericin B to deal with systemic mycosis and griseofulvin to supplement attack on dermatophytes.

Mechanism of Action

The currently available antifungal agents for the treatment of systemic mycosis include polyene antibiotics (Amphotericin B), fluoropyrimidine (Flucytosine), Nystatin and azole group of drugs (Ketoconazole, Fluconazole, Itraconazole).

Fungal diseases are called mycosis and those affecting humans can be divided into four groups based on the level of penetration into the body tissues:-

1. Superficial mycosis are caused by fungi that grow only on the surface of the skin or hair.
2. Cutaneous mycosis or dermatomycosis includes such infections as athlete's foot and ringworm, in which growth occurs only in the superficial layers of skin, nails, or hair.
3. Subcutaneous mycosis penetrates below the skin to involve the subcutaneous, connective, and bone tissue.
4. Systemic or deep mycosis is able to infect internal organs and become widely disseminated throughout the body. This type is often fatal (12).

A broad classification of antifungal drugs is given in **Table 1**.

Table 1: Classification of antifungal drugs

Class of Drug	Mechanism of Action	Example	References
Ally amines	Inhibits ergosterol synthesis by inhibiting the enzyme squalene epoxidase	Terbinafine	(12)
Antimetabolite	<ul style="list-style-type: none"> • Inhibits fungal protein synthesis by replacing uracil with 5 fluoro uracil in fungal RNA. • Inhibit thymidilate synthetase via 5-fluorodeoxy-uridinemonophosphate and thus interferes with fungal DNA synthesis. 	Flucytosine	(13)
Azoles	Inhibition of cytochrome P450 14a-demethylase (P45014DM). This enzyme is in the sterol biosynthesis pathway that leads from lanosterol to ergo sterol.	Ketoconazole	(13)
Polyenes	Act by binding to ergo sterol in the fungal cell membrane. This binding results in depolarization of the membrane and formation of pores that increase permeability to proteins and monovalent and divalent cations, eventually leading to cell death	Amphotericin B	(13)
Glucan Synthesis Inhibitors	Blocks the synthesis of a major fungal cell wall component, 1-3-beta-D-glucan.	Caspofungin	(13)
Miscellaneous	Inhibiting fungal mitosis by disrupting the mitotic spindle through interaction with polymerised spindles.	Griseofulvin	(13)

Infectious Disease

Invasive fungal infections (IFI's) are those infections where fungi have invaded into the deep tissues and have established themselves resulting in prolonged illness (14). Among the fungi that have potential to cause IFI's include Yeasts (*Candida spp*, *Cryptococcus spp*) and moulds (*Aspergillus spp*, *Fusarium spp*, *Scedosporium prolificans*, *Mucor*, *Rhizopus* and *Rhizomucor absidia*). IFI's are also caused by dimorphic fungi including *Histoplasma capsulatum*, *Coccidi oidesimmitis*, *Blastomyces dermatitidis*, *Paracoccidioides spp*, *Sporothrix spp* and *Penicillium marneffii* (15-17). Scanty reports of IFI's are also reported in literature with rare yeasts like *Saccharomyces spp*, *Trichosporon spp*, *Malassezia spp*, *Geotrichum candidum*, *Hansenula annola*, *Rhodotorula spp* and *Picchia spp* (18, 19).

Kshirsagar and co-workers modified the formulation, developed a "Patient Worthy" sterile pyrogen free liposomal amphotericin preparation and investigated it in patients with systemic fungal infections and leishmaniasis. It was found to be safe and producing significantly less adverse effects compared to plain amphotericin in patients with systemic fungal infection, did not produce nephrotoxicity and could be given to patients with renal damage. It was effective in patients resistant to fluconazole and plain amphotericin. Liposomal amphotericin was more effective than equal dose of free amphotericin B given after fungal spore challenge. A large single dose of liposomal

amphotericin was more effective, whether given before or after spore challenge, than given as two divided doses (20). Apart from this, diverse lipid based formulations of amphotericin-B are listed in **Table 2**.

Table 2: Lipid based formulations of Amphotericin

Feature	ABLC*	ABCD*	AmBisome	References
Lipid Components	DMPC*, DMPG*	Cholesteryl sulphate	Phosphatidyl choline, cholesterol, distearoyl phosphatidyl glycerol	(21, 22)
Structure	Ribbons of lipid with amphotericin B	Discoid structures with amphotericin B	Unilamellar liposomes with Amphotericin-B inside	(23)
Usual dose	5mg/kg/day	2-7.5mg/kg/day	5-7.5mg/kg/day	(23)
Acute toxicity (as compared with parent compound)	8-10 times less toxic	8-10 times less toxic	70-80 times less toxic	(24)
Safety profile	Preservation of renal function	Preservation of renal function	Adverse effects in < 5% of the patients	(25)
Efficacy response in humans	69% overall 78% candidiasis 60% aspergillosis	59% overall 83% candidiasis	67% candidiasis 86% aspergillosis	(25)

*Amphotericin B Lipid Complex (ABLC), Amphotericin B Colloidal Dispersion (ABCD), Dimyristoylphosphatidylcholine (DMPC), Dimyristoyl phosphatidyl glycerol (DMPG)

Topical Fungal Infection

The most common type of subcutaneous infection is sporotrichosis, caused by the fungus *Sporothrix schenckii*. Sporotrichosis is characterized by nodular or ulcerated lesions on areas exposed to fungal inoculation. Other examples of subcutaneous mycoses include chromomycosis and maduramycosis (or mycetoma) (25). A drug must have some specific characteristics to be delivered in the form of a topical preparation for treatment of skin fungal infections; the most important of these is its lipophilic nature. When such a drug is applied on the skin, a depot is formed in the lipidic stratum corneum which releases the drug slowly to the underlying skin layers, that is, epidermis and dermis. Therefore, in order to achieve a topical effect for an antifungal drug, the release rate of this lipophilic drug should be controlled by the formulation in order to achieve high local therapeutic concentration and to provide prolonged pharmacological effect. Another important consideration is the molecular weight of the drug; this is especially important for antifungal drugs known to exceed 500 Dalton.

Transungual Drug Delivery

The most common diseases affecting the nail are onychomycosis (fungal infections of the nail plate and/or nail bed) and psoriasis of the nails as depicted in **Figure 1**. Recent advances in topical transungual delivery had come up with antifungal nail lacquers. Current research on nail permeation focuses on altering the nail plate barrier by means of chemical treatments and penetration enhancers (26).



Figure 1: The two most common diseases affecting the nail are onychomycosis (fungal infections of the nail plate and/or nail bed) and psoriasis of the nails

New Drugs

Oxaboroles, a new class of antifungal agents, have been recently introduced. Oxaboroles penetrates the nail more effectively than ciclopirox, achieving impressive levels within and beneath the nail plate (27).

Central Nervous System Fungal Infections

Although several fungi may cause infection in normal humans, most of them are opportunistic and influence immunocompromised hosts. With the exception of *Candida albicans*, that is a normal flora of the human mucus, most fungal elements get into the body through breathing or skin scrapes.

Fungal infections involving the central nervous system are notoriously difficult to treat, and many antifungal agents have high molecular weights and a large degree of protein binding that limit their ability to penetrate the blood-brain barrier (28, 29). Of the currently available antifungal agents, 5-FC, fluconazole, and voriconazole have the best penetration in the cerebrospinal fluid and vitreous chamber of the eye (30). However, liposomal amphotericin B and perhaps other triazoles and echinocandins may still achieve concentrations in the brain parenchyma sufficient to be clinically effective (31, 32).

Causative Fungi

Cryptococcus neoformans

There are two varieties of *C. neoformans* var. *neoformans* (Serotype A and D) and var. *gatti* (Serotype B and C) (33). Invasion of the CNS with fungi can cause one or more the symptoms such as acute or chronic meningitis, abscesses or granuloma, encephalitis, stroke, parenchymal brain, or myelopathy (34, 35).

Amphotericin B and/or flucytosine in combination are recommended for CNS infections especially caused by *Candida* and *C. neoformans* (36). A high rate of morbidity and mortality of patients with fungal infections of CNS are caused by several factors, such as organ transplant, chemotherapy, ICU hospitalization, immunocompromised patients and haematological malignancies. The treatment of fungal CNS infection is influenced by multiple factors including, the host, the pathogen and its drug susceptibility, drug delivery across the blood-brain barrier and drug activity in the CNS, brain and spinal cord (37, 38).

Various Carrier Systems for Treatment of Fungal Infections

1.1. Micelles

A micelle is defined as a group of surfactant molecules dispersed in a liquid. In an aqueous solution, micellization occurs due to the mounting of hydrophilic head of surfactant molecules toward water and sequestering of the hydrophobic tails toward the inside. The ability of the micellar solution to utilize the follicular penetration pathway is utilised here. These findings suggest the promising role of micelles in improving the cutaneous bioavailability of the antifungal drugs (39).

1.2. Nanoparticles

Solid lipid nanoparticles are carriers where the drug is entrapped within a solid lipid core matrix. Examples of these lipids are triglycerides, diglycerides, monoglycerides, fatty acids, steroids, and waxes (40-42). Nanostructured lipid carriers are the second generation of lipid nanoparticles in which the matrix is composed of a mixture of solid and liquid lipids (43). Advantages of lipid nanoparticles are that the lipids utilized in their preparation are physiological lipids and can be prepared using organic solvent-free methods (44). Both solid lipid nanoparticles and nanostructured lipid carriers have been recommended as good carriers for the treatment of topical skin infections, especially for antifungal drugs which are known to be lipophilic, and hence, can be successfully entrapped within the lipidic core of solid lipid nanoparticles or nanostructured lipid carriers (45). Solid lipid nanoparticles and nanostructured lipid carriers were generally reported as effective in prolonging release of antifungal drugs and increasing their skin permeation (46).

1.3. Microemulsions

Microemulsions are defined as thermodynamically stable mixtures of oil and water stabilized by surfactants and co-surfactants, with size in the nanometer range. Owing to their ability to solubilize many poorly soluble drugs, microemulsions have been found very promising in the delivery of antifungal drugs which are characterized by their lipophilicity (47, 48).

A microemulsion gel developed for topical delivery of fluconazole for the treatment of invasive fungal infections was developed and found very effective in enhancing percutaneous absorption of the drug (49).

2. Vesicular Drug Delivery Systems

Vesicles are defined as highly ordered assemblies of one or several concentric lipid bilayers. They are formed when certain amphiphilic molecules such as phospholipids or surfactants are placed in water (50). As a topical drug carrier, vesicular systems act as penetration enhancers owing to the penetration of their lipidic components into the stratum corneum leading to alteration in the intercellular lipid matrix. They also serve as depots for localizing and sustaining the release of topically applied compounds (51, 52).

2.1. Liposomes

Liposomes are vesicles which consist of one or more concentric lipid bilayers separated by water or aqueous buffer compartments, ranging in size from 10 nanometres to 20 micrometers (53, 54). Liposomes were reported to interact with the skin via several mechanisms. They are either adsorbed onto the skin surface leading to the release of drugs, or penetrate via the lipid-rich channels either intact, or after losing some lipid lamellae; alternatively, they form occlusive films which increase skin hydration and drug penetration into the stratum corneum (55). Liposomes were also able to effectively decrease fungal colonies when encapsulating ciclopiroxolamine (56).

2.2. Transferosomes

Transferosomes, also termed as ultradeformable or flexible liposomes, have been used as carriers. They are formed of phospholipids and an edge activator; the latter is a surfactant having a high radius of curvature that destabilizes the phospholipid lipid bilayers and increases the deformability of vesicles (56).

2.3. Ethosomes

Ethosomes represent another type of deformable vesicles, which contain ethanol instead of edge activator in transferosomes as the penetration enhancer. Ethanol was reported to fluidize the intercellular lipids of the stratum corneum upon topical application and allow the easy penetration of vesicles into deeper skin layers (57). Different approaches adopted widely for delivery of anti-fungal drug are shown in **Figure 2**.

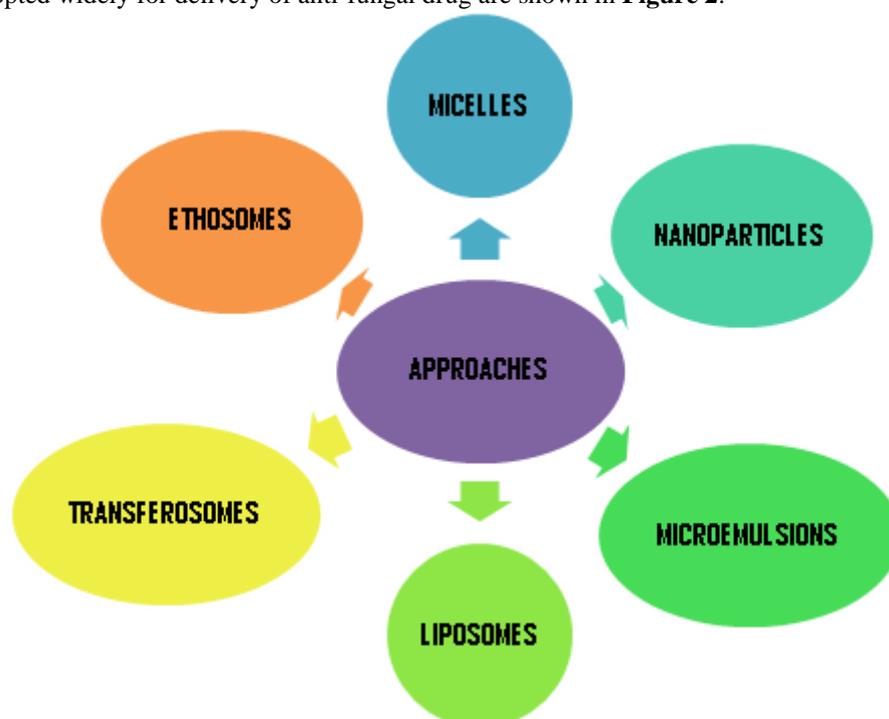


Figure 2: Novel carriers systems for treatment of fungal infection

Penetration Enhancer Vesicles

Penetration enhancer containing vesicles are a new elastic vesicular system prepared by penetration enhancers with or without soybean lecithin. Penetration enhancers differ in their chemical structure and properties, the commonly used ones being oleic acid, Transcutol and Labrasol (58).

Regarding their mechanism of action, penetration enhancer vesicles were reported to penetrate intact down to the epidermis, followed by further penetration to deeper layers owing to the enhancement of bilayers fluidity caused by the penetration enhancer (59). Oleic acid vesicles loaded with fluconazole were shown to enhance the epidermal accumulation of the drug suggesting their potential for the treatment of deep localized skin fungal infections.

Pharmacokinetic and Other Properties

Besides spectrum of activity, antifungal pharmacokinetic properties are often the most important consideration in drug selection because impaired GI tract function or reduced renal/hepatic drug clearance can profoundly influence the safety and efficacy of antifungal therapy. Several classes of antifungal agents must be administered intravenously, including amphotericin B and the echinocandins, because these agents are not sufficiently absorbed from the GI tract. Nephrotoxicity is a common dose-limiting adverse effect of amphotericin B therapy. Amphotericin B also directly stimulates release of proinflammatory cytokines by mononuclear phagocytic cells,

often resulting in fever, rigors, and chills during drug infusion (60, 61). This infusion reaction can be attenuated to varying degrees by reformulation of amphotericin B into lipid carriers. However, the principal advantage of lipid amphotericin B formulations is their reduced distribution of amphotericin B to the kidneys, which reduces but does not eliminate the nephrotoxicity of amphotericin B (62). Two formulations of amphotericin B, a liposomal formulation and a lipid complex are now commonly used to treat a wide range of invasive fungal infections.

Fluconazole and voriconazole both have oral bioavailability exceeding 90% and can be administered without regard to food (fluconazole) or preferably on an empty stomach (voriconazole) (63). Itraconazole capsules and posaconazole suspension require food to prolong gastric residence time to enhance drug dissolution, which is not an issue with the oral cyclodextrin formulation of itraconazole that is administered on an empty stomach. However, patients may prefer to take itraconazole solution with food because of GI intolerance and the unpalatable aftertaste of the solution (64).

Drug interactions are another important cause of pharmacokinetic variability because coadministration of any triazole or caspofungin with potent inducers of phase 1 (CYP) and phase 2 metabolism (i.e., rifampin, phenytoin) can potentially result in low (fluconazole, caspofungin, posaconazole) or undetectable (itraconazole, voriconazole) bloodstream concentrations of the antifungal agent and an increased risk of treatment failure. A comparison of pharmacokinetic and pharmacodynamic properties of systemic antifungal agents is given in **Table 3** and spectrum of action of systemic antifungal agents is depicted in **Figure 3**.

Table 3: Comparative pharmacokinetic and pharmacodynamic properties of systemic antifungal agents

Drug	Oral bioavailability	C _{max}	AUC	Metabolism	Elimination	t _{1/2}	References
AMB	<5	0.5-2	17	Minimal	Faeces	50	(62)
ABCD	<5	4	43	Minimal	ND	30	(62)
ABLC	<5	1.7	14	Minimal	ND	173	(62)
LAMB	<5	83	555	Minimal; urine, faeces	Minor	100-153	(62)
FLU	>90	6-20	400-800	Minor; hepatic	Renal	31	(62)
ITRA	50	0.5-2.3	29.2	Hepatic	Hepatic	24	(62)
VOR	>90	3-4.6	20.3	Hepatic	Renal	6	(62)
POS	ND	1.5-2.2	8.9	Modest hepatic	Faeces	25	(62)
ANI	<5	6-7	99	None	Faeces	26	(62)
CAS	<5	8-10	119	Hepatic	Urine	30	(62)
MICA	<5	10-16	158	Hepatic	Faeces	15	(62)
5-FC	80	30-40	30-60	Minor; intestinal	Renal	3-6	(62)

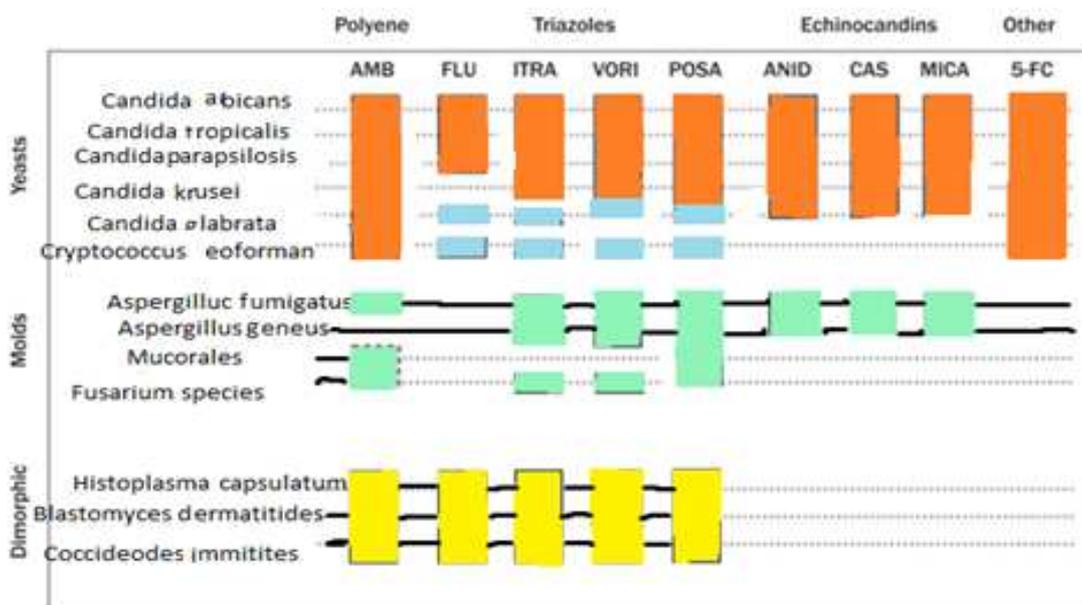


Figure 3: Spectrum of action of systemic antifungal agents. Solid blocks represent species in which the antifungal agent has demonstrated microbiological and clinical efficacy. Blocks with dotted lines indicate fungal genera/species in which resistance is common.

Finally, the site of infection is an important consideration in the selection of antifungal therapy because some antifungal agents have limited distribution to anatomically privileged sites, such as the central nervous system and vitreous fluid, or, in the case of oral itraconazole and posaconazole, may not achieve sufficient concentrations in the bloodstream to treat haematogenous infection (63- 65).

Therapeutic Drug Monitoring (TDM) of Antifungal Agents

Therapeutic drug monitoring is a process by which the drug regimen is individualized so as to maintain the drug concentration within the therapeutic range considering inter or intra subject (66). It is primarily used in measuring concentration of specific drugs in biological fluids at intervals in order to maintain a relatively constant concentration of the medication in the blood stream (67, 68).

Functions of TDM (69)

- Selection of drug
- Helps in designing dosage regimen
- Evaluate patient response
- Determine need for measuring serum drug concentrations
- Assay for drug concentration in biological fluids
- Supports the need to perform pharmacokinetic evaluation of drug concentration
- Direct for readjustment of dosage regimen if necessary
- Monitoring serum drug concentrations
- Recommend special requirements

Uses of TDM

- Allow narrow therapeutic index drug monitoring e.g. gentamycin, phenytoin, digoxin.
- Helps in improving long term therapy e.g. immunosuppressants, anti-depressant.
- GIT, hepatic or renal disease causing disturbance of drug absorption, metabolism or excretion can be monitored.
- Useful in hormone replacement therapy.
- For new drugs where no predictable dose response is there, it's crucial.
- Drug poisoning, athletic drug testing for steroids (doping) and drug abuse (for narcotics) can be checked.

Some antifungal agents exhibit marked variability in bloodstream concentrations that are difficult to predict on the basis of dosing alone, recent treatment guidelines and expert reviews have recommended therapeutic drug monitoring (TDM) for some antifungal agents in selected patient populations (70-73). Therapeutic drug monitoring has long played an important role in improving the safety of 5-FC because the drug is frequently administered with nephrotoxic agents such as amphotericin B that cause wide fluctuations in drug clearance. Bone marrow suppression and hepatotoxicity are the most common dose-limiting toxicities of 5-FC and have been strongly linked to serum peak concentrations greater than 100 µg/mL.

Toxicities of Antifungal Agents

Although the safety and tolerability of systemic antifungal therapy has improved considerably, a growing proportion of heavily immunocompromised patients are receiving systemic antifungal agents for progressively longer treatment courses. Familiar dose-limiting toxicities associated with systemic antifungal agents (i.e., infusion-related toxicities and nephrotoxicity with amphotericin B, hepatotoxicity with triazole antifungal agents) and also longer-terms risks, including recurrent drug interactions, organ dysfunction, and cutaneous reactions and malignancies should be considered before administering systemic antifungal therapy (74-76). Recent reports have linked phototoxic reaction to the subsequent development of squamous cell carcinoma and melanoma (77, 78). Although rash is reported with all antifungal classes in 5% to 15% of patients, voriconazole treatment in ambulatory patients has been associated with unique retinoid-like phototoxic reactions that present with cheilitis, erythema, and occasional blistering (79). Few examples of toxicities of antifungal agents are shown in **Figure 4**.

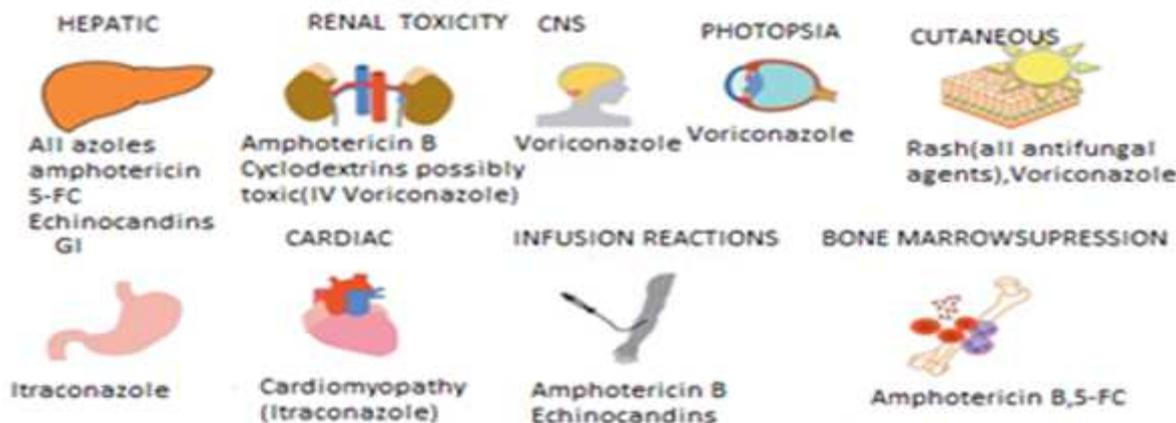


Figure 4: Common toxicities of antifungal agents (CNS: central nervous system; 5-FC: flucytosine; GI: gastrointestinal; IV: intravenous; QTc: corrected QT interval) (80)

CONCLUSION

Conventional delivery systems, including creams, ointments, and gels are traditionally being used for the treatment of skin fungal infections, even if they are deep seated. Carrier systems have the ability to overcome the immediate drug release caused by these conventional formulations, hence they avoid the possibility of induction of allergic reactions. Moreover, they are especially customized to enhance the penetration of the antifungal agents, leading to more effective treatment of skin fungal infections, especially the deeper ones. The extensive uses of corticosteroids and cytotoxic drugs and AIDS epidemic have increased the frequency of CNS mycoses. Progress of effective antifungal agents has improved the prognosis of the CNS fungal infections. Generally, antifungal drugs are highly lipophilic compounds, which can affect the penetration of drugs across *stratum corneum*. Long-term toxicities have become more of a concern because ambulatory patients with long-term immunosuppression are taking antifungal therapies for prolonged periods. For most patients, however, the benefits of safer and more effective antifungal therapy vastly outweigh the manageable risks of developing toxicity and under treating a life-threatening systemic fungal infection.

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