AN OVERVIEW ON ANTIFUNGAL THERAPY

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Summary

The number of fungi causing systemic disease is growing and the number of systemic diseases caused by fungi is increasing. The currently available antifungal agents for the treatment of systemic mycoses include polyene antibiotics (Amphotericin B), fluoropyrimidine (Flu cytosine), and Nystatin andazole group of drugs (Ketoconazole, Fluconazole, and Itraconazole). Novel drug delivery systems for antifungal therapy, based on the type of formulation are classified as Liposomes Nanocochleates, Nanospheres, Carbon Nanotubes, Doubled layered Mucoadhesive Tablets, Mucoadhesive Thermo Sensitive Pronged release gels, and Parenteral Micro emulsions. Amphotericin B is the only fungicidal agent available and is the ‘goldstandard’ for the treatment of most of the systemic mycoses. The three currently available lipid formulations are Amphotericin B Lipid Complex (ABLC), Amphotericin B Colloidal Dispersion (ABCD) and Liposomal Amphotericin B (L-AmB). Nystatin and ketoconazole are also commercially available as liposomes. Novel Drug delivery systems for antifungal therapy, aiming at reducing the side effects and maximizing the antifungal activity have added a new dimension to the treatment of fungal infections. Without fungi we would not have bread, beer, wine or antibiotics, but more importantly without the nutrient recycling and plant nutrition provided by fungi - we probably could not survive at all.

Keywords: Novel drug delivery, Antifungal, fungal infections

Introduction

Mycology is the branch of biology concerned with the systematic study of fungi, including their genetic and biochemical properties, their taxonomy, and their use to humans as a source of medicine, food, and psychotropic substances consumed for religious purposes, as well as their dangers, such as poisoning or infection.

Development of new approaches for the treatment of invasive fungal infections encompasses new delivery systems for approved and investigational compounds, as well as exploiting the cell membrane, cell wall and virulence factors as putative antifungal targets. 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.

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3. Subcutaneous mycosis penetrate below the skin to involve the subcutaneous, Connective, and bone tissue.

4. Systemic or deep mycosis are able to infect internal organs and become widely disseminated throughout the body this type is often fatal.

Systemic infection cause by fungi constitutes a major public health problem in many parts of the world. Fungi are extremely fit for survival as evidenced by their ubiquity in nature. The number of fungi causing systemic disease is growing and the number of systemic diseases caused by fungi is increasing. Systemic infections from fungi cause serious diseases, especially if septicemia develops. For people with poor immune system fungal infections continue to grow, requiring medical treatment. The currently available antifungal agents for the treatment of systemic mycosis include polyene antibiotics (Amphotericin B), fluoropyrimidine (Flu cytosine), Nystatin and Azole groups of drugs (Ketoconazole, Fluconazole, Itaconazole). The development of new antifungal drug however seems to be more difficult as compared to antibacterial due to the less target point of drug action on fungal cell as compared to that of antibacterial. Unlike bacteria, both fungi and humans are eukaryotes. Thus fungal and human cells are similar at the molecular level. This makes it more difficult to find or design drugs that target fungi without affecting human cells. The purpose of this review is to discuss the latest modern concept of antifungal therapy.

Fungal infections occur commonly in patients whose immune systems are compromised, either by an underlying disease or illness or through the use of immune-suppressing medications, often prescribed following organ transplantation or to treatment for an autoimmune disorder, such as lupus or rheumatoid arthritis. The number of diagnosed pulmonary fungal infections has grown significantly in the past decade, and diagnostic methods and treatment options have also expanded, making the need for new guidelines especially critical. "The incidence, diagnosis, and clinical severity of pulmonary fungal infections have dramatically increased in recent years in response to a number of factors. In addition to growing numbers of immune-compromised patients with HIV and other diseases, the number of patients receiving drugs to suppress the immune system following organ transplant or as the result of autoimmune inflammatory conditions has increased."

Fungal infections (mycoses) are widespread in the population specially in temperate climate they are generally associated with the skin (e.g. 'athlete's foot') or mucous membranes (e.g. 'thrush'). However, they become a more serious problem when the immune system is compromised or when they gain access to the systemic circulation. When this occurs, fungal infections can be fatal.

Fungi and fungal infections:

The cellular wall of fungi is composed of mannoproteins and chitin, which itself is composed of cellulose and hemicellulose. This is vegetable in nature and what gives the cell its rigidity. It is close to the same composition of vegetables and what causes them to stand up and grow reaching for the sunlight. The basic structural features of fungi are not cells but hyphae. Hyphae are microscopic branching threads. Each thread consists of a tube formed from a
wall enclosing cytoplasm and a vacuole. The hyphal walls are not made of cellulose but of a substance called chitin, an organic nitrogenous compound. The hyphae contain many nuclei distributed throughout the cytoplasm. Sometimes the hyphae are divided into compartments by cross walls. The fungi do not have chlorophyll so they cannot make their food in the way that plants do. They feed on dead or decaying organic matter and are classed as saprophytes. Their hyphae penetrate the dead material and form a branching network called a mycelium. The tips of the growing hyphae produce enzymes which digest the organic material. The soluble products are absorbed into the hyphae. When mould fungi, such as Rhizopus, grow on stale bread or rotting fruit, the mycelium.

Many fungi produce biologically active compounds, several of which are toxic to animals or plants and are therefore called mycotoxins. Of particular relevance to humans are mycotoxins produced by moulds causing food spoilage, and poisonous mushrooms (see above). Particularly infamous are the lethal amatoxins in some *Amanita* mushrooms, and ergot alkaloids, which have a long history of causing serious epidemics of ergotism (St Anthony's fire) in people consuming rye or related cereals contaminated with sclerotia of the ergot fungus, *Claviceps purpurea*.

Fungi are eukaryotic cells and therefore represent a more complex and evolved organism than we have hitherto considered. Thousands of fungal species, predominantly parasitic in nature, have been characterised. Many are of economic importance, either because they are useful in manufacturing other products (e.g. yeast in brewing and the production of antibiotics) or because of the damage they cause to crops or to foodstuffs. Approximately 50 are pathogenic in humans. These organisms are present in the environment or may coexist with humans as commensals without causing any overt risks to health. However, since the 1970s, there has been a steady increase in the incidence of serious secondary systemic fungal infections. One of the contributory factors has been the widespread use of broad-spectrum antibiotics, which eliminate or decrease the non-pathogenic bacterial populations that normally compete with fungi. Other causes include the spread of AIDS and the use of immunosuppressant or cancer chemotherapy agents. The result has been an increased prevalence of opportunistic infections, i.e. infections that rarely cause disease in healthy individuals. Older people, diabetics, pregnant women and burn wound victims are particularly at risk of fungal infections such as candidiasis. Primary fungal infections, rare in many parts of the temperate world, are also now encountered more often because of the increase in international travel.

Toenail fungus, also called onychomycosis, is a relatively common condition that disfigures and sometimes destroys the nail. This problem can be caused by several different types of fungi (microscopic organisms related to mold and mildew). These fungi thrive in the dark, moist and stuffy environment inside shoes. As they grow, fungi feed on keratin, the tough protein that makes up the hard surface of the toenails. In most cases, the fungus belongs to a group of fungi called dermatophytes, which include *Trichophyton rubrum* and *Trichophyton interdigitale*. Other, less common causes of onychomycosis include yeasts and molds.
Toenail fungus affects 2% to 18% of all people worldwide. It is relatively rare in children, affecting only about 1 out of every 200 people younger than 18. However, the likelihood of getting toenail fungus increases with age. Up to 48% of people have at least one affected toe by the time they reach age 70. Although 2.5 million Americans see a podiatrist annually for treatment of toenail fungus, many more are infected but never seek help. Some people consider toenail fungus just a cosmetic problem and don't bother seeking treatment. Almost anyone who wears tight-fitting shoes or tight hosiery is more likely to develop toenail fungus, especially if they also practice poor foot hygiene. Another risk is wearing layers of toenail polish, which doesn't allow the nail to breathe. Also, because toenail fungi may spread from foot to foot on the floors of showers and locker rooms, fungal infections of the toe nails are especially common among military personnel, athletes and miners. The condition also tends to affect people with chronic illnesses, such as diabetes or HIV, as well as people with circulatory problems that decrease blood flow to the toes. However, many people have no clear risk factors. When a toenail develops a fungal infection, it typically turns yellow or brown and becomes thick and overgrown. Foul-smelling debris also may accumulate under the nail, especially at the sides and tip. As the infection continues, the nail either may crumble gradually and fall off or become so thick that the affected toe feels uncomfortable or painful inside shoes. In a less common variety of toenail fungus, called white superficial onychomycosis, the nail turns white rather than yellow or brown, and the surface becomes soft, dry and powdery.

Ringworm is a skin infection caused by a fungus. Ringworm can affect skin on your body (tinea corporis), scalp (tinea capitis), groin area (tinea cruris, also called jock itch), or feet (tinea pedis, also called athlete's foot). Often, there are several patches of ringworm on your skin at once. Ringworm is a common skin disorder, especially among children, but it may affect people of all ages. Although its name suggests otherwise, it is caused by a fungus, not a worm. Many bacteria and fungi live on your body and can multiply rapidly and form infections. Ringworm occurs when a particular type of fungus grows and multiplies anywhere on your skin, scalp, or nails. Ringworm is contagious. It can be passed from one person to the next by direct skin-to-skin contact or by contact with contaminated items such as combs, unwashed clothing, and shower or pool surfaces. We can also catch ringworm from pets that carry the fungus. Cats are common carriers. The fungi that cause ringworm thrive in warm, moist areas. Ringworm is more likely when we have frequent wetness (such as from sweating) and minor injuries to your skin, scalp, or nails. Itchy, red, raised, scaly patches that may blister and ooze. The patches often have sharply-defined edges. They are often redder around the outside with normal skin tone in the center. This may create the appearance of a ring. Our skin may also appear unusually dark or light. When our scalp or beard is infected, we will have bald patches. If nails are infected, they become discolored, thick, and even crumble.

Athlete's foot is an infection of the feet caused by fungus. The medical term is tinea pedis. Athlete's foot may last for a short or long time and may come back after treatment. The body normally hosts a
variety of microorganisms, including bacteria and fungi. Some of these are useful to the body. Others may, under certain conditions, multiply rapidly and cause infections. Athlete's foot occurs when a particular type of fungus grows and multiplies in your feet (especially between your toes) or, less commonly, your hands. Of the fungal infections known as tinea infections, Athlete's foot is the most common. It may occur at the same time as other fungal skin infections such as ringworm or jock itch. These fungi thrive in warm, moist areas. Risk for getting athlete's foot increases if we wear closed shoes, especially if they are plastic-lined. Athlete's foot is contagious, and can be passed through direct contact, or contact with items such as shoes, stockings, and shower or pool surfaces. The most common symptom is cracked, flaking, peeling skin between the toes. The affected area is usually red and itchy. You may feel burning or stinging, and there may be blisters, oozing, or crusting. In addition to the toes, the symptoms can also occur on the heels, palms, and between the fingers. If the fungus spreads to your nails, they can become discolored, thick, and even crumble. Hair fungus invasion occurs on the hair shaft that the fungus grows. Common organisms in this type of infection are T. tonsurans, M. canis and M. audouinit.

PHARMACOLOGY OF ANTIFUNGAL DRUGS:

Antifungals work by exploiting differences between mammalian and fungal cells to kill the fungal organism without dangerous effects on the host. As a consequence, many antifungal drugs cause side-effects. Some of these side-effects can be life-threatening if the drugs are not used properly. Precaution should be taken during antifungal therapy as the drugs causes various side-effects like liver-damage or affecting estrogen levels, many medicines can cause allergic reactions in people. For example, the azole group of drugs is known to have caused anaphylaxis. There are also many drug interactions. Patients must read in detail the enclosed data sheet(s) of the medicine. For example, the azole antifungals such as ketoconazole or itraconazole can be both substrates and inhibitors of the P-glycoprotein, which (among other functions) excretes toxins and drugs into the intestines. Azole antifungals also are both substrates and inhibitors of the cytochrome P450 family CYP3A4, causing increased concentration when administering, for example, calcium channel blockers, immunosuppressant, chemotherapeutic drugs, benzodiazepines, tricyclic antidepressants, macrolides and SSRIs. The antifungal agents act on various targets as shown in fig 1. Drugs acting on the cell Membrane include polyene antibiotics like Amphotericin B lipid formulations, Nystatin(topical) and azole antifungals like, Ketoconazole, Itraconazole, Fluconazole, Voriconazole, Miconazole and Clotrimazole. DNA synthesis is another target for antifungal therapy, and this therapy includes drugs like Pyrimidine analogues, e.g. Flucytosine. The antifungal drugs that act on cell wall are Echinocandins, Caspofungin acetate. Table 1 gives the classification of antifungal drugs, based on their chemical structure and mechanism of action.

A number of different mechanisms contribute to the development of resistance. In the case of antifungals, they
include molecular changes in the drug target itself; overexpression of the drug target—thus swamping the antifungal agent; the reverse of overexpression, namely reduction in the concentration of the drug target—thus eliminating it as a site of action; changes in molecule biosynthesis; and pumps that actively eliminate the antifungals. Dissection of each of these mechanisms reveals new weaknesses in the pathogens, and is used as a strategy to combat the problem of resistance 47.

Fig.1. Targets for antifungal therapy

Latest approach for antifungal therapy:

Three new azole drugs have been developed, and may be of use in both systemic and superficial fungal infections. Voriconazole, ravuconazole, and posaconazole are triazoles, with broad-spectrum activity. Voriconazole has a high bioavailability, and has been used with success in immunocompromised patients with invasive fungal infections. Ravuconazole has shown efficacy in candidiasis in immunocompromised patients, and onychomycosis in healthy patients. Preliminary in vivo studies with posaconazole indicated potential use in a variety of invasive fungal infections including oropharyngeal candidiasis. Echinocandins and pneumocandins are a new class of antifungals, which act as fungal cell wall beta-(1,3)-D-glucan synthase enzyme complex inhibitors. Caspofungin (MK-0991) is the first of the echinocandins to receive Food and Drug Administration approval for patients with invasive aspergillosis not responding or intolerant to other antifungal therapies, and has been effective in patients with oropharyngeal and esophageal candidiasis. Standardization of MIC value determination has improved the ability of scientists to detect drug resistance in fungal species. Cross-resistance of fungal species to antifungal drugs must be considered as a potential problem to future antifungal treatment, and so determination of susceptibility of fungal species to antifungal agents is an important component of information in development of new antifungal agents. Heterogeneity in susceptibility of species to azole antifungals has been noted. This heterogeneity suggests that there are differences in activity of azoles, and different mechanisms of resistance to the azoles, which may explain the present lack of cross-resistance between some azoles despite apparent structural similarities. The mechanisms of azole action and resistance themselves are not well understood, and further studies into azole susceptibility patterns are required 43.

HIV patients and people receiving chemotherapy for cancer. Treatment is becoming difficult due to fungal resistance to the antifungal therapy, the variety of disease-causing fungi found and the toxic effects of conventional therapy. Scientists believe gamma interferon, a protein molecule produced by human cells in
response to infections, may help to fight fungal infections. “Immune cells called neutrophils are rapidly recruited to the site of infection and play an essential role in fungal killing. Some patients with severe asthma who also have allergic sensitivity to certain fungi enjoy great improvements in their quality of life and on other measures after taking an antifungal drug. The recently released genome sequences of Aspergillus fumigatus and Cryptococcus neoformans have provided unprecedented opportunities for comparative genomics studies of many clinically relevant fungal pathogens. Emerging experimental analysis tools, such as fitness profiling and protein microarrays, have greatly enhanced our ability to conduct genome-wide functional studies. Seven trials of antifungal agents in children with prolonged fever and neutropenia (suspected fungal infection) and candidaemia or invasive candidiasis (proven fungal infection). Four trials compared a lipid preparation of amphotericin B with conventional amphotericin B (395 participants), one trial compared an echinocandin with a lipid preparation of amphotericin B (82 participants) in suspected infection; one trial compared an echinocandin with a lipid preparation of amphotericin B in children with candidaemia or invasive candidiasis (109 participants) and one trial compared different azole antifungals in children with candidaemia (43 participants). No difference in all-cause mortality and other primary endpoints (mortality related to fungal infection or complete resolution of fungal infections) were observed. No difference in breakthrough fungal infection was observed in children with prolonged fever and neutropenia.

When lipid preparations and conventional amphotericin B were compared in children with prolonged fever and neutropenia, nephrotoxicity was less frequently observed with a lipid preparation (RR 0.43, 95% CI 0.21 to 0.90, P = 0.02) however substantial heterogeneity was observed (I² = 59%, P = 0.06). Children receiving liposomal amphotericin B were less likely to develop infusion-related reactions compared with conventional amphotericin B (chills: RR 0.37, 95% CI 0.21 to 0.64, P = 0.0005). Children receiving a colloidal dispersion were more likely to develop such reactions than with liposomal amphotericin B (chills: RR 1.76, 95% CI 1.09 to 2.85, P = 0.02). The rate of other clinically significant adverse reactions attributed to the antifungal agent (total reactions; total reactions leading to treatment discontinuation, dose reduction or change in therapy; hypokalaemia and hepatotoxicity) were not significantly different. When echinocandins and lipid preparations were compared, the rate of clinically significant adverse reactions (total reactions; total reactions leading to treatment discontinuation, dose reduction or change in therapy) were not significantly different. Limited paediatric data are available comparing antifungal agents in children with proven, probable or suspected invasive fungal infection. No differences in mortality or treatment efficacy were observed when antifungal agents were compared.

Children are less likely to develop nephrotoxicity with a lipid preparation of amphotericin B compared with conventional amphotericin B. Further comparative paediatric antifungal drug trials and epidemiological and pharmacological studies are required.
highlighting the differences between neonates, children and adults with invasive fungal infections\textsuperscript{39}.

**Novel drug delivery systems:**
Progress in the development of novel drug delivery systems is bringing researchers and clinicians closer to meeting the goals of maximum efficacy with minimal toxicity and inconvenience\textsuperscript{32}. Some drugs have an optimum concentration range within which maximum benefit is derived, and concentrations above or below this range can be toxic or produce no therapeutic benefit at all.

On the other hand, the very slow progress in the efficacy of the treatment of severe diseases, has suggested a growing need for a multidisciplinary approach to the delivery of therapeutics to targets in tissues. From this, new ideas on controlling the pharmacokinetics, pharmacodynamics, non-specific toxicity, immunogenicity, biorecognition, and efficacy of drugs were generated. These new strategies, often called drug delivery systems (DDS), are based on interdisciplinary approaches that combine polymer science, pharmaceutics, bioconjugate chemistry, and molecular biology. To minimize drug degradation and loss, to prevent harmful side-effects and to increase drug bioavailability and the fraction of the drug accumulated in the required zone, various drug delivery and drug targeting systems are currently under development. Among drug carriers, one can name soluble polymers, microparticles made of insoluble or biodegradable natural and synthetic polymers, microcapsules, cells, cell ghosts, lipoproteins, liposomes, and micelles. The carriers can be made slowly degradable, stimuli-reactive (e.g., pH- or temperature-sensitive), and even targeted (e.g., by conjugating them with specific antibodies against certain characteristic components of the area of interest). Targeting is the ability to direct the drug-loaded system to the site of interest\textsuperscript{35}.

The need for research into drug delivery systems extends beyond ways to administer new pharmaceutical therapies; the safety and efficacy of current treatments may be improved if their delivery rate, biodegradation, and site-specific targeting can be predicted, monitored, and controlled. From both a financial and a global health care perspective, finding ways to administer injectable-only medications in oral form and delivering costly, multiple-dose, long-term therapies in inexpensive, potent, and time-releasing or self-triggering formulations are also needed. The promise of administration methods that allow patients to safely treat themselves is as significant as any other health care development, particularly in developing countries where doctors, clean syringes, sterile needles, and sophisticated treatments are few and far between\textsuperscript{36}. The application of fungal genomics offers an unparalleled opportunity to develop novel antifungal drugs. As novel drug delivery systems have a great potential for modifying the pharmacokinetics of medications\textsuperscript{48}.

Novel drug delivery systems for antifungal therapy are classified as Liposomes, Nanocochleates, Nanospheres, Carbon Nanotubes, Doubled layered Mucoadhesive Tablets, Mucoadhesive Thermo sensitive Pronged release gels, and Parenteral Micro emulsions.

**Liposomes:**
A liposome is a tiny bubble (vesicle), made out of the same material as a cell membrane as shown in fig.2. Membranes are usually made up of phospholipids as shown in fig.3, which are molecules that have a head group and a tail group. The head is attracted towards water, and the tail, which is made of a long hydrocarbon chain, is repelled by water. Liposomes can be filled with drugs, and used for delivering drugs for cancer and other diseases.

Through encapsulation of drugs in a macromolecular carrier, such as a liposome, the volume of distribution is significantly reduced and the concentration of drug is increased. The particle size of liposomes varies from 20nm to 10µm. The particle size of small unilamellar vesicles (SUV) varies from 0.02-0.05µm, large unilamellar vesicles (LUV) are more than 0.06µm and multilamellar vesicles (MLV) size is in between 0.1 and 0.5µm. Liposomes have a short biological-life in blood circulation. The circulation time of liposomes in the blood stream can be increased by attaching them to polyethylene glycol (PEG)-units. The two important methods used for preparing liposomal drug delivery systems are, Simple hydration method and emulsion method.

**Fig.2. A typical liposome structure**

- Drugs delivered via liposomes may be protected from the actions of metabolizing enzymes
- Lipophilic drugs may be made soluble
- Drugs can be targeted to specific areas by attaching ligands to the liposome
- Liposomes are readily absorbed by cells. The rate of drug release may be controlled by the selection of liposome
- Using liposomes as a drug deliverer allows potentially lower doses of drug to be used, reducing
- Furthermore, it is possible that gene therapy drugs may be delivered by liposomes.

The size of the liposomes is increasingly being recognised as an important factor in treatment efficacy. The size of the liposome used in drug delivery may affect its circulation and residence time in the blood, the efficacy of the targeting, the rate of cell absorption (or endocytosis) and, ultimately, the successful release of its payload. Such size considerations are also hugely important to nanoscale polymer-encapsulated drug delivery systems.
Fig. 3. A liposome with a phospholipid molecule

The polar character of the liposomal core enables polar drug molecules to be encapsulated. Amphiphilic and lipophilic molecules are solubilized within the phospholipid bilayer according to their affinity towards the phospholipids. Participation of nonionic surfactants instead of phospholipids in the bilayer formation results in niosomes. Channel proteins can be incorporated without loss of their activity within the hydrophobic domain of vesicle membranes, acting as a size-selective filter, only allowing passive diffusion of small solutes such as ions, nutrients and antibiotics. Thus, drugs that are encapsulated in a nanocage-functionalized with channel proteins are effectively protected from premature degradation by proteolytic enzymes. The drug molecule, however, is able to diffuse through the channel, driven by the concentration difference between the interior and the exterior of the nanocage35.

Emulsion method:

AmB is a naturally occurring polyene macrolide antibiotic, produced by Streptomyces nodosus. It is the only fungicidal agent available and is the ‘gold standard’ for the treatment of most of the systemic mycosis. It acts by binding to ergosterol present in the fungal cell membrane to form ‘micropores’ or channels, thereby disrupting the membrane function and allowing electrolytes (mainly potassium) and small molecules to leak from the cell, resulting in cell death. The mechanism of action may also involve oxidative damage to the fungal cells. The usual therapeutic dose of AmB is 0.5 to 0.6 mg/kg administered by intravenous infusion. A total daily dose should not exceed 1.5 mg/kg because it has a low therapeutic index Conventional dosage forms of AmB have side effects like nephrotoxicity, chills, fever and thrombophlebitis2. Lipid formulations of Amphotericin B are needed to reduce the toxicity. Studies were initiated for introducing Amphotericin B into Lipid complex to improve the therapeutic index. The three currently available lipid formulations are Amphotericin B Lipid Complex (ABLC), Amphotericin B Colloidal Dispersion (ABCD) and Liposomal Amphotericin B (LAmB). Table 2 gives the lipid formulations of AmB.

Amphotericin B lipid complex (ABLC):

AmB is complexed with dimyristoyl phosphatidylcholine (DMPC) & dimyristoyl phosphatidylglycerol (DMPG)3. The configuration is ribbon like and is tightly packed with Amb along with lipid, which reduces the toxicity, and achieves lower plasma levels than conventional AmB, hence ABLC is less toxic & more effective. Clinical success rate of 40% was observed in 151 definite or probable invasive aspergillosis cases.
with ABLC and the corresponding rate determined by retrospective analysis was 23% in 122 patients treated with conventional AmB. The licensed dose is 7mg/kg.

**Amphotericin B colloidal dispersion (ABCD):**

It is a disk-like structure formed by Amphotericin B combined with cholesteryl sulfate in a 1:1 molar ratio. The formation of this complex leaves no free AmB and achieves high concentration in liver. Nephrotoxicity of ABCD is less than parent compound AmB due to low Serum levels and less LDL bound to AmB. The usual dose 3-6mg/kg and high dose of 7.5mg/kg/day is safely used. This colloidal dispersion has been approved by the US FDA and is given by IV route.

**Liposomal Amphotericin B (L-AmB):**

It is composed of AmB, hydrogenated soy phosphatidylcholin distearoylphosphatidylcerol and cholesterol. It is a true liposome composed of unilamellar lipid vesicles with an average size of 60-70 nm. L-AmB has high plasma concentration and longer circulation time than other lipid formulations. The usual dose is 1-5mg/kg. Table 3 gives the commercially available lipid formulations.

**Nystatin:**

Brown and Hazen discovered Nystatin in 1949 in soil samples containing a strain of *Streptomyces* noursei. It was licensed for use in 1951 for superficial Candida infections of the oropharynx, esophagus, and intestinal tract. Metha et al reported that liposomal nystatin & free nystatin have same minimum inhibitory concentration (MIC). Oakley et al tested 60 species of Aspergillus with Nystatin and liposomal nystatin, and found lower minimum inhibitory concentration (MIC) for liposomal Nystatin. A study by Johnson et al showed that in vitro activity of L-nystatin and nystatin were less than that of AmB and ABLC and were more potent than LAMB. L-nystatin is currently in phase III clinical trials, is administered by IV route in doses of 0.25-4mg/kg. L-nystatin is given by IV route in doses of 0.25-4mg/kg.

**Nanocochleates:**

Cochleates are cigar-like microstructures that consist of series of lipid bilayers, formed as a result of condensation of small unilamellar negatively charged liposomes. The small phosphatidylserine (PS) liposomes fuse in the presence of Calcium ions (Ca²⁺) and form large sheets. These sheets have a hydrophobic surface and tend to roll-up into the cigar-like cochleates to minimize the interaction with water. The hydrophobic and hydrophilic surfaces of these sheets are suitable for encapsulating both hydrophobic drugs like AmB and Clofazimine and amphipathic drugs like doxorubicin respectively.

**Preparation of nanocochleates of Amphotericin B:**

AmB and L-a-dioleoylphosphatidylserine were dissolved in suitable solvents in a molar ratio of 10:1 of lipid:drug. The solvent was evaporated, followed by addition of water, resulting in the formation of a suspension, which on sonication yielded small AmB liposomes.

**Nanospheres:**
Systemic Candidiasis is associated with high mortality and prolonged hospitalization\textsuperscript{26}. Treatment with potent drugs like AmB causes severe toxic effects. Nanospheres of AmB with natural carriers as Sodium alginate were found to be effective in terms of optimum drug loading capacity. The nanosphere bound drug may enhance drug localization. Table 5 gives the AmB - loading efficiency of sodium alginate nanospheres\textsuperscript{26}.

**Preparation of nanospheres:**
Calcium chloride and sodium alginate were used to induce gelling in the suspension.

0.1\%w/v solution of Poly-l-lysine was added to it to form a polyelectrolyte complex. Suspension was stirred continuously and kept overnight for stabilization. Separation was done by ultracentrifugation and dried under vacuum to form flaky mass, with an average particle size of 419.6 ± 0.28nm which on redispersion in sterile WFI produced discrete particles \textsuperscript{26}. Table 5 gives the AmB - loading efficiency of sodium alginate nanospheres.

**Carbon nanotubes:**
Nanotubes possess a unique feature of being able to enter the living cell without causing death or damage. The mechanism by which they pass through the cell membrane is not clear\textsuperscript{27}. Amphotericin B is covalently attached to carbon nanotube, thus preventing its aggregation. The incorporation of AmB on nanotube reduces the dose of drug, since the activity is enhanced after conjugation with nanotubes. The stability and flexibility of carbon nanotubes is likely to prolong the circulation time and bioavailability of macromolecules and thus enabling effective drug therapy.

**Doubled layered mucoadhesive tablets:**
Buccoadhesive drug delivery systems have been developed, basically to increase the retention of the drug in oral cavity. These are designed for the treatment of oral Candidiasis. Double layered tablets of Nystatin were prepared by direct compression technique using lactose, Carbopol and hydroxypropyl methyl cellulose (HPMC)\textsuperscript{18}.

**Vaginal gel:**
The objective of vaginal gel formulation with thermosensitive and mucoadhesive properties is to ensure longer residence time and provide desired release profile with. β cyclodextrin complex\textsuperscript{20}. Cyclodextrins are used to form inclusions with drug molecule for decreasing side effects\textsuperscript{31, 22}.

**Preparation of gel\textsuperscript{20}:**
Mucoadhesive polymer (Carbopol 934 or HPMC) was dissolved in Citrate phosphate buffer (0.1M, pH 4.0) with gentle mixing. Pluronic F 127 was added to the buffer and dissolved. Clotrimazole in free form was dissolved in a mixture of PEG 400 and Ethyl alcohol and added to cold Pluronic F 127 solution containing the mucoadhesive polymer.

**Advantages:**
Mucoadhesive property ensures longer residence time at the site of application, due to complex formation\textsuperscript{20}. Controlled release of drug can be achieved, ensuring antimycolytic efficacy.
for longer period, better patient compliance and higher therapeutic efficacy.

**Parenteral microemulsions:**

Microemulsions are clear, stable, isotropic liquid mixtures of oil, water and surfactant, frequently in combination with a co-surfactant. The aqueous phase may contain salt(s) and/or other ingredients, and the "oil" may actually be a complex mixture of different hydrocarbons and olefins. Parenteral formulation of itraconazole as an o/w microemulsion system has better therapeutic index than AmB.

**Conclusion:**

Novel Drug delivery systems for antifungal therapy have less toxic effects and more antifungal activity compared to their parent compounds given by conventional systems. The development of lipid based antifungal agents has opened a new era in the treatment of fungal infections. Advances in liposome technology will hopefully result in more efficient and less toxic antifungal regimens. The fungus causes diseases in plants as well as animals, but they are also very useful for breaking down dead organic matter which allows nutrients to be cycled through the ecosystem. Many plants cannot grow without the fungi that inhabit their roots and supply them with essential nutrients, they are of economic importance for the production of bread, beer, wine and antibiotics (e.g. yeast)

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Table 1: Shows Classification of Antifungal Drugs:

| Class of Drug | Mechanism of Action | Example |
|---------------|---------------------|---------|
| Ally amines   | Inhibits ergo sterol synthesis by inhibiting the enzyme squalene epoxidase | Terbinafine |
| Antimetabolite| Inhibits fungal protein synthesis by replacing uracil with 5 fluoro uracil in fungal RNA, also inhibit thymidilate synthetase via 5-fluorodeoxy-uridine monophosphate and thus interferes with fungal DNA synthesis. | Flu cytosine |
| Azoles        | Inhibition of cytochrome P450 14a-demethylase (P45014DM). This enzyme is in the sterol biosynthesis pathway that leads from lanosterol to ergo sterol | Ketoconazole |
| 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 |
### Glucan Synthesis Inhibitors

| Miscellaneous | Blocks the synthesis of a major fungal cell wall component, 1-3-beta-D-glucan. Inhibiting fungal mitosis by disrupting the mitotic spindle thru interaction with polymerized microtubules | Caspofungin, Griseofulvin |

### Table 2: Shows Lipid Formulations of Amphotericin

| Feature                      | ABLC                        | ABCD                        | AmBisome                                      |
|------------------------------|-----------------------------|-----------------------------|------------------------------------------------|
| Lipid Components             | DMPC, DMPG                  | Cholesteryl sulphate        | Phosphatidyl choline                          |
|                              |                             |                             | Cholesterol, distearoyl phosphatidyl glycerol. |
| Structure                    | Ribbons of lipid with amphotericin B | Discoid structures with amphotericin B | Unilamellar liposomes with amphotericin B inside. |
| Acute toxicity (as compared with parent compound) | 8-10 times less toxic. | 8-10 times less toxic. | 70-80 times less toxic. |
| Usual dose                   | 5mg/kg/day                  | 2-7.5mg/kg/day              | 5-7.5mg/kg/day                                |
| Safety profile               | Preservation of renal function. | Preservation of renal function. | Adverse effects in < 5% of the patients        |
| Efficacy response in humans  | 69% overall                | 59% overall                | 67% candidiasis                              |
|                              | 78% candidiasis             | 83% candidiasis             | 86% aspergillos                              |
|                              | 60% aspergillos             |                             |                                                |
| Trade name                   | Abelcet                     | Amphocil                    | AmBisome                                      |
| DMPC: Dimyristoyl phosphatidylcholine; DMPG: Dimyristoyl phosphatidylglycerol |

### Table 3: shows commercially available lipid formulations.

| Parent compound             | Vehicle Lipid configuration | Lipid formulation     | Commercial name                      |
|-----------------------------|-----------------------------|-----------------------|--------------------------------------|
| Amphotericin B              | DMPC, DMPG                  | Ribbon-like           | ABLC                                |
| Amphotericin B              | Cholesteryl sulfate         | Disk-like             | ABCD                                 |
| Amphotericin B              | HSPC, DSPG, Cholesterol     | Vesicle(ULV)          | Liposomal amphotericin B             |
| Amphotericin B              | EPC, triglycerids Glycerol  | Undefined             | Amphotericin B 20% fat emulsion      |
| Amphotericin B              |                              |                       | Intralipid                           |
| Nystatin                    | DMPC, DMPG                  | Vesicle(MLV)          | Liposomal Nystatin                   |
| Nystatin                    |                              |                       | Nyotran                              |
| Hamycin                     | DMPC, DMPG,                 | Vesicle(MLV)          | Liposomal                            |
|                             |                             |                       | --------                             |
Table 4: Shows Pharmacokinetics properties of Amphotericin B and its Lipid Formulations in Humans\(^1\)

| Drug (mg/kg) | Cmax  | AUC 0-24h | Volume of Distribution |
|-------------|-------|-----------|------------------------|
| AMB (0.6)   | 1.1 ± 0.2 | 17.1 ± 5  | 5 ± 2.8                |
| ABLC (5)    | 1.69 ± 0.75 | 11.9 ± 2.6 | 131 ± 7.7              |
| ABCD (5)    | 3.1    | 43        | 4.3                    |
| L-AMB (5)   | 46     | 269 ± 96  | 0.22 ± 0.17            |

Table 5: Shows AmB loading efficiency of sodium alginate nanospheres\(^26\)

| Formulation code | Drug concentration (µg/ml) | Drug loading (%) |
|------------------|----------------------------|------------------|
| ASA I            | 10                         | 10.7 ± 0.2       |
| ASA II           | 20                         | 13.5 ± 0.6       |
| ASA III          | 30                         | 17.2 ± 0.8       |
| ASA IV           | 40                         | 22.6 ± 0.4       |
| ASA V            | 50                         | 27.3 ± 0.7       |

ULV: Unilamellar Vesicles; MLV: Multilamellar Vesicles

ASA-Amphotericin B Sodium Alginate nanosphere