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Review Article

NOVEL DRUG DELIVERY SYSTEMS FOR ANTIFUNGAL THERAPY

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ABSTRACT

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), Nystatin and azole 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 'gold standard' 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.

Keywords: Novel drug delivery, Antifungal

INTRODUCTION

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.
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 caused 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 group of drugs (Ketoconazole, Fluconazole, Itraconazole).The purpose of this review is to discuss some of the novel approaches in the management and therapy of fungal diseases.

Targets for antifungal therapy

The antifungal agents act on various targets. 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. Flu cytosine. 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.

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.. Membranes are usually made up of phospholipids, 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.

The major structural components of liposomes are Phospholipids and Cholesterol as shown in fig 1. 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 multi lamellar 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.

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 erg sterol 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 thrombophlebitis [2]. 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 (L-AmB). 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[4]. The licensed dose is 7mg/ kg. [3]

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 [5] and achieves high concentration in liver [6]. 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 [7, 8, 9, 10] and high dose of 7.5mg/kg/day is safely used [11, 12]. 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 phosphatidylcholine, distearoylphosphatidylglycerol, and cholesterol [13]. 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 [1].

Nystatin

Brown and Hazen discovered Nystatin in 1949 in soil samples containing a strain of *Streptomyce*s 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 L-AMB. L-nystatin is currently in phase III clinical trials and is administered by I V route in doses of 0.25-4mg/kg [14, 15] and has fewer side effects than nystatin.

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 (Ca^{2+}) and forms 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[17], 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 [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 [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 [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 [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) [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[20]

Cyclodextrins are used to form inclusions with drug molecule for decreasing side effects [21, 22].

Preparation of gel [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[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 [29] as an o/w micro emulsion 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.

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 fluro 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	Blocks the synthesis of a major fungal cell wall component, 1-3-beta-D-glucan.	Caspofungin,
Miscellaneous	Inhibiting fungal mitosis by disrupting the mitotic spindle thru interaction with polymerized microtubules	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 78% candidiasis 60% aspergillosis	59% overall 83% candidiasis	67% candidiasis 86% aspergillosis
Trade name	Abelcet	Amphocil	AmBisome

DMPC: Dimyristoyl phosphatidylcholine: DMPG: Dimyristoyl phosphatidylglycerol

Table 3: shows commercially available lipid formulations.

Parent compound	Vehicle Lipid	Lipid configuration	Lipid formulation	Commercial name
Amphotericin B	DMPC, DMPG	Ribbon-like	ABLC	Abelcet
Amphotericin B	Cholesteryl sulfate	Disk-like	ABCD	Amphocil, Amphotec
Amphotericin B	HSPC, DSPG , Cholesterol	Vesicle(ULV)	Liposomal amphotericin B	AmBisome
Amphotericin B	EPC,triglycerids Glycerol	Undefined	Amphotericin B 20% fat emulsion	Intralipid
Nystatin	DMPC, DMPG	Vesicle(MLV)	Liposomal Nystatin	Nyotran
Hamycin	DMPC, DMPG, Cholesterol	Vesicle(MLV)	Liposomal hamycin	--------
Miconazole	EPC, HSPC	Vesicle (ULV& MLV)	Liposomal miconazole	--------
Ketoconazole	EPC, HSPC	Vesicle (ULV & MLV)	Liposomal Ketoconazole	--------

ULV: Unilamellar Vesicles: MLV: Multilamellar Vesicles

Table 4: Shows Pharmacokinetics properties of Amphotericin B and its Lipid Formulations in Humans. [1]

Drug (mg/kg)	Cmax	AUC 0-24 h	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

ASA-Amphotericin B Sodium Alginate nanosphere

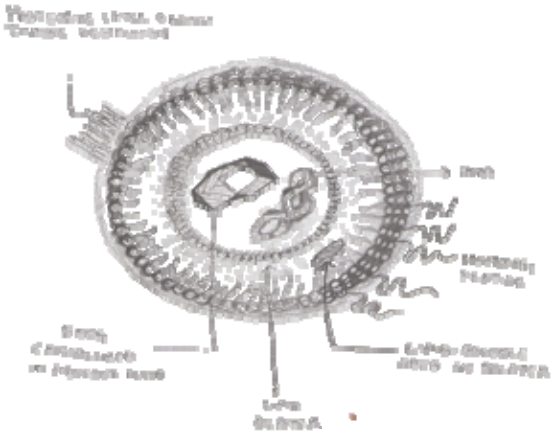

Fig 1: Shows structure of Liposome

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