Biochemical Targets for Antifungal Chemotherapy

Fungal cells are complex organisms that share many biochemical targets with other eukaryotic cells. Therefore, agents that interact with fungal targets not found in eukaryotic cells are needed.

The fungal cell wall is a unique organelle that fulfills the criteria for selective toxicity.

Fungal cell wall differs greatly from bacterial cell wall. Therefore, fungi are unaffected by antibacterial cell wall inhibitors such as β-lactams and vancomycin.
Biochemical Targets for Antifungal Chemotherapy

Arrangement of the biomolecular components of the cell wall accounts for the individual identity of the organism. Although, each organism has a different biochemical composition, their gross cell wall structure is similar.

Antifungal agents targeted towards:

**Inhibition of fungal cell wall synthesis** – caspofungin is a β-glucan synthesis inhibitor; several more compounds are under investigation

**Inhibition of fungal cell membrane synthesis** – ergosterol is the target (cell membranes of fungi and mammals contain different sterols): polyenes, azoles, triazoles, alkylamines

**Inhibition of cell division** – microtubule effects: griseofulvin; DNA: flucytosine.
Antifungal Agents - Sites of action

- **Echinocandins**
  - Inhibit fungal cell wall biosynthesis

- **Polyenes**
  - Integration into cell membrane

- **5-Fluorocytosine**
  - Interruption of DNA & RNA synthesis

- **Azoles**
  - Interruption of sterol biosynthesis (cell and mitochondrial membranes)

- **Griseofulvin**
  - Inhibits mitotic spindle formation

- **Echinocandins**
  - Inhibit fungal cell wall biosynthesis

- **Cell wall**
- **Plasma membrane**
- **Mitochondrion**
Antifungal Agents

Unless indicated all are on the UW formulary

1. Polyene Antifungal Drugs

These drugs interact with ergosterol in the fungal cell membrane and form pores

Polyenes are related chemically to the macrolide antibiotics with the large lactone ring but have the distinctive characteristic of conjugated double bonds and a lipophilic (a chromophore of 4-7 conjugated double bonds) and hyrophilic side (several alcohols, acids and usually a sugar).

The number of conjugated double bonds correlates directly with antifungal activity in vitro and inversely with the degree of toxicity to mammalian cells. They are unstable, only slightly soluble, and poorly absorbed when taken orally.

• Amphotericin B
• Nystatin
• (Natamycin) Pimaricin
Mechanism of Action of Polyenes

Polyenes bind to fungal membrane sterols. The selective effect is achieved because the sterol in highest concentration is ergosterol and polyenes have a high affinity for ergosterol. They insert into the membrane and disrupt membrane function. The membranes become leaky.

Ergosterol is not present in mammalian membranes. Recent thinking is that the polyenes form small transmembrane pores that allow K to leak through. See figure next slide. The polyenes are fungicidal at high concentrations.
Mechanism of Action of Polyenes
Amphotericin B, a polyene antibiotic, is produced by *Streptomyces nodosus*. Discovered in 1956 has been for 30 years the main available drug to control serious fungal infections. Amp. B is indicated for treatment of severe, potentially life threatening fungal infections.

Unfortunately, it must be given IV and is toxic (due to nonselective action on cholesterol in mammalian cell membranes). Serious fungal infections involve long therapy.

Resistance is due to lower production of membrane sterols or altered sterols, but is relatively rare at present. Target modification and reduced access to target are other mechanisms of resistance.
Amphotericin B

Disposition

Amp. B is not absorbed orally. It is given as a colloidal dispersion by slow IV infusion. It is highly bound to cholesterol-lipoprotein and has a plasma T1/2 of about 1 day and 1-2 weeks from tissues. It is excreted in urine over a long time.

Penetration into the CNS is poor. However, for fungal infections of the CNS, amphotericin B is mixed with cerebrospinal fluid (CSF) that is obtained from a spinal tap. The solution of amphotericin is then reinjected through the tap.
Amphotericin B

Adverse Effects:
1) Reactions on infusion - headache, fever, chills, anorexia, vomiting, muscle and joint pain. Pain at site of injection and thrombophlebitis are frequent complications of intravenous administration. Drug must never be given intramuscular. Can give aspirin, meperidine, steroids, antiemetics etc to prevent some of these.

2) Nephrotoxicity - chronic renal tox in up to 80% of patients taking the drug for prolonged periods. It is reversible but can be irreversible in high doses. Test for kidney function regularly. This is the most common limiting toxicity of the drug.

3) Hematologic - hemolytic anemia due to effects on RBC membrane.

4) Other less common reactions - cardiac, convulsions, neuropathy, hearing loss, allergic, etc.
   Some decrease in adverse effects particularly nephrotoxicity with liposomal preparations; the idea with the lipid preps is to decrease nonspecific binding to mammalian membranes.
Products:

**Amphotericin B.** (Fungizone ®) 50 mg/vial with 41mg of sodium deoxycholate. Reconstitute with water. Give a test dose and gradually increase dose. Don't exceed 1.5mg/kg/d. Alternate day therapy is sometimes used. Several months of therapy is usually needed.

**Abelcet** (Liposome Co.) 1:1 mixture of amphotericin and lipid complex, 100 mg/20 ml. Rationale for this lipid preparation is that amphotericin B should have a greater affinity for the lipid vehicle than for cholesterol in cell membranes, thus lower toxicity. Lipid associated amphotericin B is drawn into the reticuloendothelial system, concentrating in lymphatic tissues, spleen, liver and lungs where infectious fungi concentrate. Lipases excreted from fungi release drug from lipid carrier allowing to bind to ergosterol in fungal cell membranes to exert fungistatic and fungicidal activities.

**Aphotec** (Sequus Pharmaceuticals) cholesteryl colloidal dispersion, 50 or 100 mg/20 ml (not on UW formulary) Supplied in variety of topical forms including a 3% cream, lotion or ointment and 100mg/mL oral suspension to treat cutaneous and mucocutaneous mycoses caused by *Candida albicans*

**AmBiosome** (Fujisawa) liposomal, 50mg/vial.
Nystatin

Isolated from streptomyces noursei in 1951. A conjugated tetraene, is the first clinically useful polyene antifungal antibiotic. Available in oral tablets, powder for suspension, vaginal tablets, pastilles. This polyene is used for local therapy only (not absorbed). For gut Candidiasis, and in a "swish and swallow" routine for oral Candidiasis.

No significant adverse effects with these uses. Combined with tetracycline to prevent monilial overgrowth caused by the destruction of bacterial microflora of the intestine during tetracycline therapy.

(Mycostatin ® and other generic products)
Natamycin (Pimaricin; Natacyn)

Polyene antibiotic obtained from cultures of Streptomyces natalensis. Structures consists of 26-membered lactone instead of the 38 for Nystatin and Amphotericin B. The 26-membered polyenes cause both K leakage and cell lysis at same concentration.

Natamycine supplied as a 5% ophthalmic suspension intended for the treatment of fungal conjunctivitis, blepharitis and keratitis.
Azole Antifungal Agents

Azole antifungal agents are the largest class of synthetic antimycotics. About 20 agents on the market today. Some used topically to treat superficial dermatophytic and yeast infections. Others used systemically to treat severe fungal infections. Antifungal activity stems from the presence of an aromatic five member heterocyclic, either an imidazole (two nitrogen atoms) or a triazole (three nitrogen atoms).

The first members of the class were highly substituted imidazoles (clotrimazole, miconazole) were not absorbed orally. Ketoconazole introduced in 1984 was the first effective oral therapy for Candida.

Itroconazole and Fluconazole are more potent, less toxic and provide effective oral therapy for many systemic fungal infections. These two are triazoles.

Another triazole has been recently introduced (voriconazole).

That said, amphotericin B is usually the preferred drug for life threatening systemic fungal infections. It is still the “gold standard”.
Examples of Azole agents

Miconazole
Econazole
Oxiconazole
Sulconazole
Tioconazole
Ketoconazole
Fluconazole
Itraconazole
Voriconazole
Specific Azole Agents

1) Ketoconazole (KCZ) - fairly broad spectrum, PO antifungal. Most of the use of this drug for significant fungal infections has been replaced by fluconazole and itraconazole.

2) Fluconazole- (FCZ) Oral and IV. It is indicated for candidiasis (oral, esophageal, vaginal) and for Cryptococcus infections including Cryptococcal meningitis. It also is being used for other fungal infections. It is used in a low dose (50-100 mg/d) to prevent candidiasis and cryptococcal meningitis in AIDS patients. It is used as a one time stat dose (150 mg) for vaginal candidiasis. It is an expensive drug but has relatively few adverse effects.

3) Itraconazole (ICZ) - Oral and IV, also a suspension. Introduced in late 1992. Is indicated for a number of systemic infections. Also for oral and esophageal candidiasis. Also for dermatophytic infections of the toenail and fingernail (Tinea unguium). It has broad antifungal activity.

4) Voriconazole (VCZ)- Oral and IV. Introduced in 2002. At present is indicated for invasive Aspergillus and several other serious invasive fungal infections, e.g. Fusarium sp. Taken one hour before or after a meal. Highly bioavailable.
Drug interactions with Azoles

**Ketoconazole** - significant inhibitor of several P450 isozymes. Fairly selective for CYP3A4. Increases levels of a number of drugs taken concurrently. Severe nephrotoxicity with cyclosporine A. Must reduce cyclosporine A dose. Rifampin induces metabolism of ketoconazole and visa versa. Will interact with any drug mainly cleared by CYP3A4.

**Fluconazole** – CYP3A4 inhibitor but less potent than KCZ. Minor effect on cyclosporin although potential is there. Inhibits CYP2C9 and 2C19 therefore potential interactions with warfarin and phenytoin. Other drugs may show increased levels. Rifampin induces metabolism of FCZ.

**Itraconazole** – Inhibits CYP3A4 and CYP2C9 but less potent than KCZ. In high doses should reduce dose of cyclosporin. The potential for elevated levels of other drugs metabolized by CYP3A4 taken concurrently is significant. Rifampin - same as above.

**Voriconazole** – similar drug interactions as ICZ.
Newazole agent more recently approved

Posaconazole (Schering-Plough)

Novel trizole antifungal recently approved for use as an oral suspension to treat invasive fungal infections. Fungistatic against Candida and fungicidal against Asperigillus species.

Similar structure to Itraconazole, absorption greatly affected by food. Mainly metabolized by phase II glucuronide conjugation and has little interaction with P450 enzymes.
Nucleoside Antifungals

Orally active antifungal with a very narrow spectrum of activity

Flucytosine was synthesized in 1957 as an antitumor agent. It was inactive but it was found to have antifungal activity. Drug inters fungal cell through active transport on ATPases that normally transport pyrimidines. Once inside cells, fungal cytosine deaminase convert the drug to active 5-fluorouracil (5FU) which is incorporated into RNA causing faulty RNA synthesis. It is also is a strong, non-competitive inhibitor of thymidylate synthesis interrupting the one carbon pool substrate. Mammalian cells do not contain cytosine deaminase.

\textbf{Flucytosine (5-Fluorocytosine)}

\begin{align*}
\text{Flucytosine} & \quad \text{cytidine deaminase} \quad \text{5-Fluorouracil} \\
\end{align*}
Nucleoside Antifungals

Resistance develops rapidly and occurs on many levels e.g. transport into the cell and cytosine deaminase steps. After a few dosing intervals the drug is essentially useless. To avoid rapid resistance, combination with Amphotericin B, and the combination is synergistic. It is also synergistic with itraconazole and fluconazole, and interest in these combinations for treatment of systemic Candida infections is increasing. Amphotericin B damaged membranes are thought to allow better entry of flucytosine.

Used (with Amp. B) for Cryptococcal meningitis, systemic Candida infections, and some other systemic fungal infections.

**Adverse Effects**

1) GI upset - very common.

2) Hepatic - 5% have increased transaminases.

3) Hematologic - anemia, leukopenia, thrombocytopenia; this is the major complication of therapy and may be due to low levels of 5-FU circulating.

4) Adverse effects seen when plasma levels reach >100 mcg/ml
Ergosterol Biosynthesis Inhibitors

Allylamines

Have a more limited spectrum of activity than the azoles and are only effective only against dermatophytes. Employed in treatment of fungal infections of skin and nails.

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\text{Inhibits squalene epoxidase (not a P450 enzyme) involved in conversion of squalene to squalene-2,3-expoxide decreased squalene-2,3 epoxide leads to decreased lanosterol and ergosterol. This decrease alters the physical-chemical properties of the membrane resulting in pH imbalance, malfunction of membrane embedded proteins. Inhibition of Squalene epoxidase results in accumulation of squalene which in itself is toxic to fungal cells. May be fungicidal.}
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Terbinafine

Lamisil® Novartis, 250 mg tabs
Echinocandins, a group of cyclic peptides with long lipophillic sidechains have been under investigation for a number of years. They interfere with cell wall biosynthesis through inhibition of the enzyme β-1,3-glucan synthase. Reduction of in the glucan content weakens the cell wall and leads to rupture of fungal cells. Some agents made it up to phase III trials only to fail due to formulation problems.
Echinocandins

Caspofungin *(parenteral)*

approved for invasive aspergillosis in patients refractory to or intolerant of other therapies. IV use only

Micafungin

Anidulafungin
**Miscellaneous Antifungals**

**Griseofulvin**

Antifungal antibiotic produced from *Penicillium griseofulvin*. Effects on microtubules to inhibit cell division microsize and ultramicrosize.

Therapy must continue until new tissue replaces old diseased tissue. When given orally, plasma-borne griseofulvin becomes incorporated into keratin precursor cells and ultimately into keratin which cannot then support fungal growth.

Griseofulvin is mainly effective on dermatophytes.

Headache is a common adverse effect. May cause aplastic anemia. Being gradually replaced by newer agents.
Undecylenic Acid

H2C=CH(CH2)8COOH

Widely used as the zinc salt in OTC preparations for topical treatment of infections by dermatophytes. A fungistatic acting through non-specific interaction with components in cell membrane.

Can be used in concentrations up to 10% in solution, powder and emulsions. Traditionally used for athlete’s foot (tinea pedis) although cure rates are low.

Ciclopirox

A hydroxylated pyridinone used for superficial dermatophytic infections mainly onychomycosis. It caused inhibition of polyvalent cations (Fe+3) and caused inhibition of metallic enzymes in the fungal cell. A new formulation of an 8% lacquer has been recently introduced for treating nail infections.
Antifungal Agents (Re-cap)

- **Echinocandins**: Inhibit fungal cell wall biosynthesis

- **Griseofulvin**: Inhibits mitotic spindle formation

- **5-Fluorocytosine**: Interruption of DNA & RNA synthesis

- **Azoles**: Interruption of sterol biosynthesis (cell and mitochondrial membranes)
Antifungal agents under development

All inhibitors of fungal cell walls

a) Other β-1,3 glucan synthetase inhibitors
   Papulacandins – glycolipid antifungal produced by Papularia sp.

b) Chitin Synthase inhibitors  Polyoxins and Nikkomycins–
   nucleoside peptides

c) Mannan binding antifungals  Pradimicins and benanomicins