VI. SYSTEMIC ANTIFUNGAL AGENTS

A. Fungal Pathogens

The fungal kingdom includes yeasts, molds, rusts and mushrooms. In general most fungi are beneficial and are involved in biodegradation. A few can cause opportunistic infections if they are introduced into a human through wounds or by inhalation.

The importance of fungi as pathogens is increasing due to aging population, HIV, immunosuppression in organ transplant and unknown factors.

Three antifungal drugs currently in the top 200:

- Fluconazole (Diflucan)
- Terbinafine (Lamisil)
- Itraconazole (Sporanox)

Mycoses

a) Superficial – caused by a variety of fungi, especially the dermatophytes (causing infection of the skin, hair and nails) belonging to 40 related fungi of three genera: Microsporum, Trichophyton, Epidermophyton. Dermatophytic infections known as Tinea and are named for the site of infection rather than the causative organism.

Examples:

<table>
<thead>
<tr>
<th>Disease</th>
<th>Etiology</th>
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<tbody>
<tr>
<td>Tinea corporis</td>
<td>Microsporum canis, Trichophyton mentagrophytes</td>
</tr>
<tr>
<td>(ringworm)</td>
<td></td>
</tr>
<tr>
<td>Tinea pedis</td>
<td>T. rubrum, T. mentagrophytes, Epidermophyton floccosum</td>
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<tr>
<td>(athlete’s foot)</td>
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<tr>
<td>Tinea cruris</td>
<td>T. rubrum, T. mentagrophytes, E. floccosum</td>
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<tr>
<td>(jock itch)</td>
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<tr>
<td>Tinea capis</td>
<td>M. canis T. tonsurans</td>
</tr>
<tr>
<td>(scalp)</td>
<td></td>
</tr>
<tr>
<td>Tinea barbae</td>
<td>T. rubrum, T. mentagrophytes</td>
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<tr>
<td>(beard/hair)</td>
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<tr>
<td>Tinea unguium</td>
<td>T. rubrum, T. mentagrophytes, E. floccosum</td>
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<tr>
<td>(nails)</td>
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</table>

Tinea unguium, whether of fingernails or toenails, can be difficult to treat because the fungi invade the nail itself.

b) Subcutaneous mycoses – variety of fungi involved. Infection starts with trauma inoculation from soil or plants.

c) Systemic mycoses – regional infections initiated by inhalation of spores; not transmitted human to human. Infections occur in otherwise healthy individuals. Not common.

Coccidioides immitis - a soil fungus that causes coccidiomycosis. The infection is endemic in the SW USA and parts of Latin Am. Is usually a self limiting lung infection but may disseminate if cellular immunity is impaired. Disseminated disease involves many organs and tissues and CNS.
**Histoplasma capsulatum** - soil fungus causing an intracellular mycosis of the reticulo-endothelial system. Central and Eastern USA.

**Blasomyces dermatitidis** - fungus found especially in N. America. Starts in lungs and may disseminate to skin and other sites.

**Paracoccidioides brasiliensis** - systemic mycosis in S. Am.

d) Opportunistic mycoses – all are exposed but immunocompromised are at high risk

**Candida albicans** - a yeast that is found in low numbers as part of the normal flora of the GI tract and vagina. If disseminated, it can cause thrombophlebitis, endocarditis, and involvement in other organs such as the lungs and kidneys.

Infection of the mouth (thrush), female genitalia (vulvovaginitis) (monilia), skin and nails are common. This yeast is becoming a very significant pathogen and is now the fourth leading cause of bloodstream infections (after staph aureus, staph epi, and enterococcus sp).

Other yeasts – other Candida species and even Saccharomyces sp can cause problems. Other Candida species are increasing in importance as pathogens, e.g. Candida glabrata. These may be resistant to fluconazole.

**Cryptococcus neoformans** - another yeast. Found in bird feces. Not normally part of flora. Gets in via inhalation and may cause a mild lung involvement. If inhaled in large doses or if in immunocompromised host (AIDS), may result in systemic disease, commonly meningitis in AIDS.

**Aspergillus** sp. - a mold. Causes aspergillosis. May grow in lung, brain, sinuses, or other organs in some immunosuppressed, 70-100% mortality (with Rx!)

Mucormycosis – caused by *Mucor* sp and related fungi – sinuses, eyes, blood, brain

**Pneumosystis carinii** – previously thought to be a protozoa. Pneumonia in AIDS

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**B. 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 vancomycins.
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.

**Fungal cell wall inhibition** – caspofungin is a β-glucan synthesis inhibitor; several more compounds are under investigation

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

**Cell Division** – microtubule effects: griseofulvin; DNA: flucytosine.

C. ANTIFUNGAL DRUGS (unless indicated, all are on UW formulary)

1. Polyenes: membrane disrupters- Amphotericin B and Nystatin

   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 hydrophilic 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.

**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.

Natamycin supplied as a 5% ophthalmic suspension intended for the treatment of fungal conjunctivitis, blepharitis and keratitis.
Amphotericin B

a) History and Structural Characteristics:

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.

b) Mechanism of Action:

These agents 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 has two conjugated double bonds that is lacking in mammalian membrane steroids (mainly cholesterol). See structures below. 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 below. The polyenes are fungicidal at high concentrations.
c) **Spectrum**

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.

d) **Resistance**

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.

Antifungal resistance to date generally involves emergence of naturally resistant species. No data to suggest that antibiotic modification in an important antifungal resistance mechanism.
**e) 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.

**f) 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.

**g) 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.

2. **Imidazoles and Triazoles**
a) Structural Features and History

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 or a triazole.
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.

Structure activity studies revealed that the imidazole ring can be replaced by a bioisosteric triazole ring without affecting the antifungal activity but achieving higher selectivity of the fungal targets vs. host.

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”.

b) Mechanism of Action:

These imidazoles and triazoles inhibit CYP P450 14 α- demethylase in fungi. This enzyme is involved in the conversion of lanosterol to ergosterol. Other P450's in sterol biosynthesis may be affected. The basic nitrogen of the azole ring forms a tight bond with the heme iron of the fungal P450 preventing substrate and oxygen binding. Inhibition of the C14α-demethylase results in accumulation of sterols still bearing a C14 methyl group changing the exact shape and physical properties of the membrane causing permeability changes and malfunction of membrane imbedded proteins.

They have a lower affinity for mammalian P450's. The effect is fungistatic, but may be fungicidal at higher concentrations.

c) Spectrum and Uses (Systemic Therapy Only)

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.

d) **Resistance**

Primary mode or resistance due to development of mutations in the active site of C14-α-sterol demethylase protecting the heme in the enzyme pocket from binding to azoles but allowing lanosterol (natural substrate) to bind. Cross-resistance is conferred to all azoles.

Increased azole efflux by sterol transporters and increased production of C14-α-demethylase can be other modes or resistance.

e) **Disposition**

1) Absorption -
   - KCZ - Need acid for dissolution. Avoid concurrent antacids or use of H2 blockers or omeprazole.
   - FCZ - no special problems; highly bioavailable
   - ICZ - take with food as food increases AUC substantially
   - VCZ – highly bioavailable

2) **Distribution**

   - KCZ and ICZ - highly lipophilic and highly protein bound. Not great CSF levels.
   - FCZ and VCZ - gets into CNS and may be used for meningitis

3) Elimination -

   - KCZ, ICZ and VCZ - extensively metabolized by 2C9, 2C19, 3A4; 2C19 exhibits genetic polymorphism and slow metabolizers (15-20% of Asian populations) may need dose reduction. No dosage adjustment in renal failure needed. ICZ hydroxy metabolite is active.
   - FCZ - Eliminated largely unchanged in urine. Adjust dose if renal problems.

f) **Adverse Effects**

   KCZ - mild hepatotoxicity in about 10%. Severe fatal hepatotoxicity in about 1/10,000. The usual GI upset in 3-10%. Hormonal effects. Due to inhibition of mammalian androgen synthesis, get gynecomastia, oligospermia, decreased libido, and menstrual problems. Testosterone levels are lowered at doses of 800 mg/d. Some use in prostate cancer.

   FCZ - a remarkably well tolerated antifungal. A few reports of severe hepatotoxicity and about 1% show an increase in transaminases. Not as potent an inhibitor of P450 as KCZ. Pregnancy category C.

   ICZ - nausea in 10%, rash in 9%, 3% show elevated transaminases; a few reports of severe hepatotoxicity. Some reports of congestive heart failure. Not as potent a P450 inhibitor as KCZ. Pregnancy category C.

   VCZ – prolonged use may cause visual impairment (~11%) which seems to be reversible. Some increase in liver transaminase. Pregnancy category D.

g) **Drug Interactions**

   KCZ - significant inhibitor of several P450 isozymes. Fairly selective for CYP3A4. Increased levels of a number of drugs taken concurrently. Severe

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

ICZ – 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.

VCZ – similar drug interactions as ICZ.

h) Products

Ketoconazole - Nizoral® Janssen, tabs.
Fluconazole - Diflucan® Roerig, injection and suspension.
Itraconazole - Sporanox® Janssen, capsules, oral solution, and injection. Take with food.
Voriconazole – Vfend® Roerig, tablets and injection.

FIG. 2. Ergosterol biosynthetic pathway. Steps at which various antifungal agents exert their inhibitory activities are shown. TERB, terbinafine; FLU, fluconazole; ITRA, itraconazole; VOR, voriconazole.
Posoconazole (Schering-Plough)

Novel trizole antifungal in Phase 3 clinical trials to be used 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. Expected approval 2005.

3. Nucleoside Antifungals: Flucytosine (5-Fluorocytosine, 5-FC)

Orally active antifungal with a very narrow spectrum of activity.

a) History and Structure and MOA

Flucytosine (5-Fluorocytosine)

Flucytosine was synthesized in 1957 as an antitumor agent. It was inactive but it was found to have antifungal activity. Drug enters 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 and also is a strong, non competitive inhibitor of thymidylate synthesis interrupting the one carbon pool substrate. Mammalian cells do not contain cytosine deaminase.

\[ \text{dUMP} \xrightarrow{\text{dTMP}} \]
b) **Spectrum and Uses**

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. This is an oral drug. Good also for fungal UTI.

c) **Disposition**

The drug is well absorbed and well disturbed. CSF levels are 65-90% of plasma levels. Is eliminated unchanged largely in the urine.

d) **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

4. **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

**Terbinafine**

![Terbinafine Structure]

Lamisil ® Novartis, 250 mg tabs

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

**Absorption**
- well absorbed > 70%

**Distribution**
- Highly plasma protein bound

**Metabolism**
- Extensively metabolized
- Cleared via the urine

**Indications**
- Treatment of onychomycosis of the toenail or fingernail due to dermatophytes.

**Warnings**
- monitor CBC in patients receiving treatment for > 6 months
- monitor hepatic function in patients receiving long term therapy
- note: now has a “boxed warning” on rare hepatotoxicity; maybe 1/50,000 treated
- pregnancy category B; no significant drug interactions

**Dose**
- 1 tab/d for 6 weeks for fingernails
- 1 tab/d for 12 weeks for toenails
5. Cell wall inhibitors

Interference with fungal cell wall biosynthesis has not been as successful and effective as penicillins and cephalosporins against bacteria. Many chemicals have been discovered which interfere with various steps in fungal cell wall synthesis with excellent antifungal activity in vitro. Unfortunately, development of these agents into useful drugs has proven very difficult.

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 -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.

Caspofungin acetate Cancidas ® Merck

Recently approved for invasive aspergillosis in patients refractory to or intolerant of other therapies

Semisynthetic lipopeptide (echinocandin) derived from a fermentation product of Glarea lozoyensis; IV use only

Mechanism of action
• (1,3)-D-glucan synthesis inhibitor
• no evidence of antagonism with amphotericin B. What about synergy?
• is fungicidal

Spectrum of activity
A. fumigatus, A. flavus, A. terreus; a recent study showed caspofungin to be at least as effective as amphotericin B for invasive Candidiasis.
Pharmacokinetics
- plasma concentrations decline in a triphasic manner
  - α-phase (short)
  - β-phase (half-life 9-11 hours)
  - γ-phase (half-life 40-50 hours)
- the dominant mechanism influencing plasma clearance is distribution
- approximately 97% protein bound to albumin
- slowly metabolized by hydrolysis, N-acetylation, and spontaneous chemical degradation; the metabolites are excreted in the urine and the feces, and only a small amount of unchanged caspofungin (approximately 1.4%) is excreted in the urine.
- No dosage adjustment is necessary based on gender, age, race or renal insufficiency. Not dialyzable. Dosage should be adjusted for hepatic insufficiency.

Side effects
- thrombophlebitis, vein irritation
- histamine-related symptoms (rash, facial swelling, pruritis); anaphylaxis has been reported
- increased LFT’s, especially in patients receiving cyclosporine

Drug interactions
- poor substrate for cytochrome P450 enzymes; does not inhibit CYP3A4
- reduces AUC, Cmax, and C of tacrolimus
- cyclosporine increases AUC of caspofungin
- other inhibitors/inducers may affect caspofungin concentrations

Pregnancy category C

Dosing: 70mg (over 1 hr) on day 1, then 50mg IV qd (mix in normal saline, not dextrose)

6. 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.
Headache is a common adverse effect. May cause aplastic anemia. Being gradually replaced by newer agents.

CYP 3A4 inducer

**Dose:** daily for many months

7. **Organic acids:**

**Undecylenic Acid**

\[
\text{H}_2\text{C}==\text{CH(CH}_2)_8\text{COOH}
\]

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.

8. **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 Nikkommucins—nucleoside peptides

c) Mannan binding antifungals
   Pradimicins and benanomicins