# ORAL CANDIDAL INFECTIONS AND ANTIMYCOTICS

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**ABSTRACT:** The advent of the human immunodeficiency virus infection and the increasing prevalence of compromised individuals in the community due to modern therapeutic advances have resulted in a resurgence of opportunistic infections, including oral candidoses. One form of the latter presents classically as a white lesion of "thrush" and is usually easily diagnosed and cured. Nonetheless, a minority of these lesions appears in new guises such as erythematous candidosis, thereby confounding the unwary clinician and complicating its management. Despite the availability of several effective antimycotics for the treatment of oral candidoses, failure of therapy is not uncommon due to the unique environment of the oral cavity, where the flushing effect of saliva and the cleansing action of the oral musculature tend to reduce the drug concentration to sub-therapeutic levels. This problem has been partly circumvented by the introduction of the triazole agents, which initially appeared to be highly effective. However, an alarming increase of organisms resistant to the triazoles has been reported recently. In this review, an overview of clinical manifestations of oral candidoses and recent advances in antimycotic therapy is given, together with newer concepts, such as the post-antifungal effect (PAFE) and its possible therapeutic implications.

**Key words.** Antimycotics, *Candida*, oral candidosis, post-antifungal effect (PAFE).

## (I) *Introduction*

Candidosis is by far the most common oral fungal infection in man and manifests in a variety of clinical guises. The main reason for the high incidence of oral candidosis in man appears to be the multiplicity of predisposing factors which aid and abet the conversion of commensal *Candida* to a parasitic existence. For instance, it is known that, in the West, a majority of elderly denture-wearers suffer from *Candida*-associated denture stomatitis by virtue of the prostheses being constantly worn in the mouth (Budtz-Jørgensen *et al.*, 1975; Samaranayake, 1990; Kuc *et al.*, 1999). Further, the advent of the human immunodeficiency virus (HIV) infection has resulted in a resurgence of oral candidal infections. Thus, it has been reported that more than 90% of HIV-infected individuals develop oral candidosis during some point of their disease; it is by far the most common oral manifestation in these patients (Samaranayake and Holmstrup, 1989; Feigal *et al.*, 1991; Arendorf *et al.*, 1998). The existence of these individuals, together with other compromised patient groups in the community, along with the usage of broad-spectrum antibiotics and corticosteroids, and common endocrine disorders such as diabetes mellitus—all have resulted in a resurgence of oral candidosis as a relatively common affliction (Samaranayake, 1990).

Yeasts which belong to the genus *Candida* are imperfect unicellular dimorphic fungi which multiply, primarily, by the production of buds from ovoid blastospores or blastoconidia; they also form hyphae and/or pseudohyphae, depending on environmental conditions (Fig. 1). In general, from 50 to 60% of humans carry *Candida* in the oral cavity (Samaranayake and Lamey, 1988), although there may be some geographic variations (Sedgley *et al.*, 1997).

*C. albicans* is the most common *Candida* species residing in the oral cavity, in both health and disease, and is the agent of most oral candidal infections (Samaranayake and MacFarlane, 1990; Zegarelli, 1993; Silverman *et al.*, 1996). All forms of oral candidoses are considered opportunistic, and hence the epithet "the disease of the diseased" given to this condition (Samaranayake and MacFarlane, 1990). Perusal of recent literature reveals that oral manifestations of candidal infections could be many and varied. These range from erythematous variants, linear gingival erythema associated with HIV infection, to median rhomboid glossitis, possibly of multi-factorial origin. In this review, we attempt to describe these entities, in some depth, together with approaches to management, especially in relation to antifungal therapy.

Several effective antifungal agents are available for the management of oral candidosis (Garber, 1994; Greenspan, 1994), and they are administered either topically or systemically. These agents range from the clas-

sic polyenes to the azole groups. Newer antifungal agents which are in the experimental and trial stages include amorolfine, natifine, terbinafine, tolaftate (McGinnis and Rinaldi, 1996), rilopirox (Braga *et al.*, 1996), cilofungin, pradimycin (Samaranayake and Ferguson, 1994), voriconazole (Ruhnke *et al.*, 1997), and benanomicin A (Ohtsuka *et al.*, 1997). These will not be discussed in detail in this review.

Despite the availability of such a multiplicity of agents, therapeutic failure is not uncommon (Samaranayake and MacFarlane, 1981). In general, treatment of fungal infections is considerably more difficult than treatment of bacterial infections. Many fungal infections occur in the poorly vascularized tissues or avascular structures such as the superficial layer of the mucosae, skin, nails, and hair. Management of these poses a therapeutic problem due to the poor solubility, distribution, and retention of adequate amounts of some of these drugs at the site of infection (Finch and Snyder, 1994). In the case of the mouth, the diluent effect of saliva and the cleansing action of the oral musculature, in particular the tongue, often tend to reduce the availability of the antifungal agents below the effective therapeutic concentrations (Martin, 1989, 1990). Further, *Candida* biofilms on oral and inert surfaces such as prostheses may also contribute to failure of drug therapy (Hawser and Douglas, 1995). Finally, poor patient compliance due to the need for frequent drug administration and associated adverse effects, coupled with a possible underlying immunodeficiency, can also impair therapy, resulting in chronic recurrence of the disease.

What follows is a description of the clinical manifestations of oral candidosis together with common therapeutic agents that are used in the management of oral candidosis. In addition, susceptibility testing of antimycotics and newer concepts such as the post-antifungal effect (PAFE) and their impact on the management of oral candidosis will be addressed.

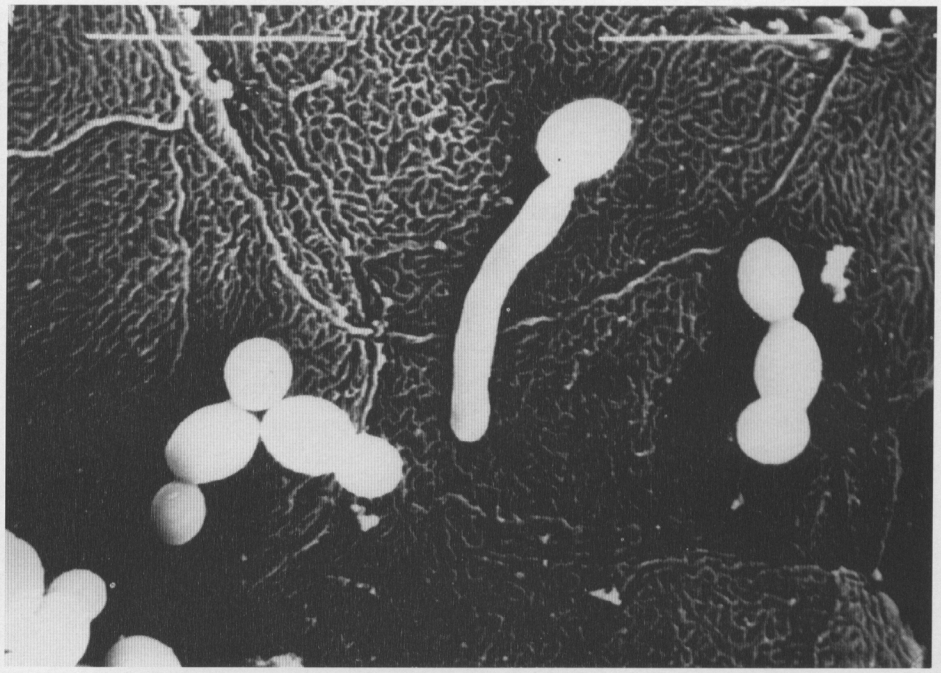

**Figure 1.** Scanning electron micrograph illustrating blastospores, budding yeast cells, and hyphae of *C. albicans* attached to buccal epithelial cells.

## (II) Candida *and Clinical Manifestations of Oral* Candida *Infections*

The fungal infections of the oral mucosa most frequently encountered are those due to species of the genus *Candida*, and C. *albicans* is the principal species associated with this infection (Samaranayake and MacFarlane, 1990), but non-*albicans* species such as *tropicalis*, *glabrata*, *parapsilosis*, and *krusei* are also pathogenic to man. The latter three species are considered emerging pathogens and are reviewed in detail elsewhere (Weems, 1992; Samaranayake and Samaranayake, 1994; Fidel *et al.*, 1999). C. *dubliniensis* is a recently described species associated with oral lesions in HIV-infected individuals and is closely related to C. *albicans*, both phenotypically and genotypically (Sullivan *et al.*, 1995).

*Candida* is present as a commensal in the oral cavities of up to 40% of healthy individuals. The number of organisms in the saliva of carriers increases in pregnancy, with tobacco smoking, and when dentures are worn (Samaranayake, 1996). The primary oral reservoir for the organism in carriers is the dorsum of the tongue, where it is usually found as blastospores. There is an overlap in the candidal counts from carriers and from individuals showing infection, and hence isolation of *Candida* from the mouth of an adult is not confirmatory evidence of infection and must be considered in tandem with the clinical findings (Samaranayake and MacFarlane, 1990; Soames and Southam, 1993).

*Candida* species are notorious opportunistic pathogens (Odds, 1994), and both general and local predisposing factors are important in the pathogenesis of oral candidal infections. For instance, debilitated patients such as those receiving broad-spectrum antibiotics, steroids, or cytotoxic therapy, patients with diabetes mellitus, xerostomia, severe nutritional deficiencies, and immunosuppressive diseases such as AIDS are more prone to oral candidosis (Samaranayake, 1991). Further local factors, such as trauma from unhygienic or

**TABLE 1**

**Classification of Oral Candidosis**
[as Proposed by Samaranayake (1991) and Modified by Axéll *et al.* (1997)]

| Primary Oral Candidoses* | Secondary Oral Candidoses |
|---|---|
| Acute forms | Oral manifestations of systemic mucocutaneous candidosis as a result of diseases such as a thymic aplasia and candidosis endocrinopathy syndrome |
| Pseudomembranous | |
| Erythematous | |
| Chronic forms | |
| Hyperplastic | |
| Nodular | |
| Plaque-like | |
| Erythematous | |
| Pseudomembranous | |
| *Candida*-associated lesions | |
| Denture stomatitis | |
| Angular cheilitis | |
| Median rhomboid glossitis | |
| Keratinized primary lesions superinfected with *Candida* | |
| Leukoplakia | |
| Lichen planus | |
| Lupus erythematosus | |

* Note: *Candida* may also contribute to the development of linear gingival erythema (LGE) and necrotizing periodontal disease in HIV infection.

ill-fitting dentures and tobacco smoking, are also important (Scully *et al.*, 1994). Nevertheless, it is often difficult to identify the exact predisposing factor, despite intensive investigation (Samaranayake and Lamey, 1988).

Classification of oral *Candida* infections is fraught with difficulties due to the varied manifestations of the disease. It is generally accepted that oral candidosis can be divided into two broad categories, *i.e.*, primary and secondary oral candidosis (Samaranayake and Yaacob, 1990; Table 1). Thus, candidal infections confined to oral and peri-oral tissues are considered primary oral candidoses, and disorders where oral candidosis is a manifestation of generalized systemic candidal infections are categorized as secondary oral candidosis (Samaranayake, 1996; Axéll *et al.*, 1997). The primary oral candidoses are subclassified into three major variants, *viz.*: pseudomembranous, erythematous, and hyperplastic, each of which may manifest as acute or chronic lesions. The essential difference between the newer and the old classifications is that the former attempts to define the lesions more in terms of clinical rather than histopathologic criteria (Axéll *et al.*, 1997). Despite such attempts at drawing a distinction between disease variants, there are conditions—such as cheilo-candidosis and chronic multifocal candidosis—which do not fall strictly into any of these categories. The former presents as a chronic, ulcerative granulating lesion of the vermilion area of the lower lip, while the latter causes chronic, erythematous plaque-like lesions in two or more sites in either the mouth, palate, or dorsum of the tongue (Samaranayake and Lamey, 1988).

*Candida* and oral candidosis have been previously reviewed elsewhere (Samaranayake and MacFarlane, 1990; Odds, 1994; Scully *et al.*, 1994). Discussed below is a brief account of the variants of oral candidosis.

### (A) Pseudomembranous candidosis

Classically, this is an acute infection, but it may recur for many months or even years in patients using corticosteroids topically or by aerosol, and in immunocompromised patients. It may also be seen in neonates and among terminally ill patients, particularly in association with serious underlying conditions such as leukemia and other malignancies, and is increasingly seen in HIV disease.

Thrush is characterized by white patches on the surfaces of the oral mucosa, tongue, and elsewhere. The lesions form confluent plaques that resemble milk curd and can be wiped off to reveal a raw, erythematous, and sometimes bleeding base (Samaranayake, 1996).

Esophageal candidosis, or candidosis of the upper respiratory tract, is a possible complication of oro-pharyngeal candidosis. The combination of oral and esophageal candidosis is particularly common in HIV infection.

### (B) Erythematous candidosis

Previously known as antibiotic sore mouth, and the atrophic variant, erythematous candidosis is associated with chronic usage of corticosteroids, broad-spectrum antibiotics, and, more commonly, with HIV disease. It may arise as a consequence of persistent acute pseudomembranous candidosis, when pseudomembranes are shed, may develop *de novo*, or, in HIV infection, may precede pseudomembranous candidosis. It is the most common variant of candidosis seen in HIV disease (Samaranayake and Holmstrup, 1989).

Clinically, erythematous areas are seen on the dorsum of the tongue, palate, or buccal mucosa. Lesions on the dorsum of the tongue present as depapillated areas. Red areas often seen in the palate in HIV disease are thought to be due to reduced epithelial thickness and/or increased vascularity. There can be an associated angular stomatitis.

### (C) Hyperplastic candidosis (*Candida* leukoplakia)

*Candida* leukoplakias are chronic, discrete raised lesions that vary from small, palpable, translucent, whitish areas to large, dense, opaque plaques, hard and rough

on palpation (plaque-like lesions). They may present as homogenous or speckled lesions (nodular lesions) which do not rub off. *Candida* leukoplakias usually occur on the inside surface of one or both cheeks at the commissural areas and less often on the tongue. Since the condition is pre-malignant and shows various degrees of dysplasia, biopsy is important. The risk of malignant transformation in candidal leukoplakia (approximately 15%) depends on whether the lesion is homogenous or speckled, the presence and degree of epithelial dysplasia, and the management adopted (Walker and Arendorf, 1990). Iron and folate deficiency and defective cell-mediated immunity have been associated with *Candida* leukoplakias in a few cases.

In addition to the foregoing three major variants of oral candidosis, there are several other lesions associated with *Candida* infection, as described below.

### (D) *Candida*-ASSOCIATED DENTURE STOMATITIS (DENTURE-INDUCED STOMATITIS, DENTURE SORE MOUTH, CHRONIC ATROPHIC CANDIDOSIS)

The characteristic presenting features of denture-induced stomatitis are chronic erythema and edema of the mucosa that comes into contact with the fitting surface of the denture; this condition is mainly due to the overgrowth of commensal strains of C. *albicans* beneath the dentures (Mathaba *et al.*, 1995). The mucosa beneath the mandibular dentures is hardly ever involved. Apart from soreness, this condition is usually symptomless. The only presenting complaint may be an associated angular stomatitis. A few patients have complained of a burning or tingling sensation beneath the denture.

Denture-induced stomatitis has been classified into three clinical types: type I, a localized simple inflammation or a pinpoint hyperemia; type II, an erythematous or generalized simple type presenting as more diffuse erythema involving a part of, or the entire, denture-covered mucosa; and type III, a granular or papillary type commonly involving the central part of the hard palate and alveolar ridge (Newton, 1962). It was thought that the papillary types of lesions are essentially due to the presence of the prostheses. However, a recent report of similar lesions in HIV infection implies that the denture is not the prime etiologic factor for the condition (Reichart *et al.*, 1997).

The presence of dentures can also cause changes in the microbial flora and accumulation of plaque between the mucosal surface of the denture and the palate. Thus, denture-induced stomatitis is most often caused by the accumulation of microbial plaque on and in the fitting surface of the denture and the underlying mucosa. It is not exclusively associated with *Candida*. Other factors—such as bacterial infection, mechanical irritation, or an allergic reaction to the denture base material—have also been implicated (Budtz-Jørgensen, 1990).

### (E) ANGULAR STOMATITIS (PERLECHE, ANGULAR CHEILITIS)

Angular stomatitis affects the angles of the mouth, characterized by soreness, erythema, and fissuring, and is commonly associated with denture-induced stomatitis (Budtz-Jørgensen, 1990). Both yeasts and bacteria (especially *Staphylococcus aureus*) are involved, as interacting and predisposing factors (Budtz-Jørgensen, 1990; Warnakulasuriya *et al.*, 1991; Dias and Samaranayake, 1995). Angular stomatitis may present as an isolated feature of iron deficiency anemia or vitamin B12 deficiency, which may resolve once the underlying disease has been treated. It may also be seen in patients with HIV disease (Samaranayake and Holmstrup, 1989). It is likely that the lesion is a result of skin maceration at deep, occlusive folds of the skin at the angles of the mouth in individuals with reduced facial height caused by old age or ill-fitting dentures.

Exfoliative chelitis, predominantly of the lower lip, may be associated with *Candida*, especially in HIV infection, and could be considered another variant of candidosis in AIDS patients (Reichart *et al.*, 1997).

### (F) MEDIAN RHOMBOID GLOSSITIS

Median rhomboid glossitis is characterized by an area of papillary atrophy that is elliptical or rhomboid, symmetrically placed at the midline of the tongue, anterior to the circumvallate papillae. Occasionally, it presents with a hyperplastic exophytic or even lobulated appearance. The relevance of *Candida* to the etiology of the condition has been controversial (Walker and Arendorf, 1990). A mixed bacterial and fungal flora may be associated with the condition (Scully *et al.*, 1994).

### (G) CHRONIC MULTIFOCAL ORAL CANDIDOSIS

This term has been given to chronic candidal infection that may be seen in multiple oral sites, with various combinations, including: (1) angular stomatitis, which is unilateral or bilateral and associated with denture wearers; (2) retro-commissural leukoplakia; (3) median rhomboid glossitis; and (4) palatal lesions. Additional criteria may include: (1) lesions of more than four weeks' duration; (2) an absence of predisposing medical conditions; and (3) exclusion of patients who had received radiotherapy or any of the following drugs: antibiotics, anti-inflammatory or immunosuppressive drugs, and cytotoxic or psychotropic agents (Holmstrup and Bessermann, 1983).

At the time of presentation, most patients are adult male tobacco-smokers, in their fifth or sixth decade. Though antifungal therapy would clear the infection and

produce clinical improvement, recurrence is common, unless smoking can be reduced.

### (H) Oral candidosis associated with systemic infections

Candidosis is usually restricted to the skin and mucous membranes but may occasionally spread. Systemic forms of candidosis may affect only one organ or be disseminated hematogenously (Odds, 1994). It is important to recognize that oral candidal infections can also manifest as a result of such systemic disease. However, most of these complications, with the exception of acquired immunodeficiency syndrome (AIDS), are rare; the candidosis remains superficial, and patients usually do not die from disseminated candidosis. Interestingly, a case of *Candida*-associated zygomatic osteomyelitis has been reported in a diabetic patient with oral candidosis (Arranz-Caso *et al.*, 1996).

### (I) Candidosis and the immunocompromised host

A few patients have chronic candidosis from an early age, sometimes with a definable and heritable immune defect (*e.g.*, chronic mucocutaneous candidosis). However, the number of patients immunocompromised by disease such as HIV infection, by hematological malignancy, and by drug therapies such as aggressive cytotoxic therapy has increased during recent years and constitutes by far the largest immunocompromised group in the community.

#### (a) Chronic mucocutaneous candidosis (CMC)

CMC is the term given to the group of rare syndromes, sometimes with a definable immune defect, in which there is a persistent mucocutaneous candidosis that responds poorly to topical antifungal treatment (Kirkpatrick, 1993). In general, the more severe the candidosis, the greater the likelihood that immunological defects can be identified. Recent studies suggest that a defect in cytokine (interleukin-2 and gamma interferon) production in response to candidal and some bacterial antigens, along with reduced serum levels of IgG2 and IgG4, may be a major cause for the infection (Lilic *et al.*,1996).

#### (b) HIV-related oral candidosis

Ever since the first clinical definition of AIDS in 1981, the CDC/WHO have recognized candidosis of the mouth as one of the major opportunistic infections and an important indicator of HIV infection. For instance, more than 90% of HIV-infected patients suffer from oral candidosis at some point in the course of the disease (Samaranayake and Holmstrup, 1989; Feigal *et al.*, 1991; Greenspan, 1994), and it is by far the most common fungal infection in HIV disease (Begg *et al.*, 1996; Arendorf *et al.*, 1998). Oral carriage of *Candida* species is also increased in these patients (Scheutz *et al.*, 1997). Further, immunocompromised persons are also at particular risk from deep mycoses which may cause chronic oral ulceration, bizarre mouth lesions, or maxillary sinus infection (Scully *et al.*, 1997).

The main cause of oral candidosis in HIV infection is immune impairment. However, *Candida* itself may also induce immunosuppression, and this can influence the prognosis of HIV infection (Odds, 1994).

The manifestations of candidal infections in HIV infection are usually restricted to superficial candidosis with various degrees of severity. The major clinical variants of oral candidosis—namely, pseudomembranous, erythematous, and hyperplastic candidoses—have all been described in HIV-infected individuals (Kolokotronis *et al.*, 1994; Silverman *et al.*, 1996). Pseudomembranous candidosis may involve any area of the oral mucosa, but most frequently the tongue, hard and soft palate, and buccal mucosa.

The erythematous form of candidosis may be a common early oral manifestation of HIV infection and presents as a pink or red macular lesion, typically on the palate and dorsum of the tongue, often mixed with white lesions (Kolokotronis *et al.*, 1994; Silverman *et al.*, 1996). Papillary hyperplasia is occasionally seen in the palate (Reichart *et al.*, 1994).

*Candida* may also contribute to the development of linear gingival erythema (LGE) or necrotizing periodontal diseases in HIV-infected persons (Odden *et al.*, 1994; Grbic *et al.*, 1995).

## (III) *Treatment of* Oral *Candidosis*

Antifungal agents that are available for the treatment of candidosis fall into three main categories: the polyenes (nystatin and amphotericin B); the ergosterol biosynthesis inhibitors — the azoles (miconazole, clotrimazole, ketoconazole, itraconazole, and fluconazole), allylamines, thiocarbamates, and morpholines; and the DNA analogue 5-fluorocytosine (White *et al.*, 1998). The historical development and introduction of these agents for therapeutic use over the last half century are summarized in Table 2 (Sheehan *et al.*, 1999). However, the principal antifungals used against oral mycoses belong to the polyenes and the azoles (Epstein, 1990; Samaranayake, 1996; Table 3).

### (A) Polyene antifungal agents

Two polyenes—namely, amphotericin B and nystatin—are commonly used for the treatment of oral candidosis. Though fungal resistance to these agents is rare/low (McGinnis and Rinaldi, 1996; White *et al.*, 1998), significant resistance in yeasts, including C. *albicans*, has been

**TABLE 2**

**Key Events in Antifungal Drug Development** (Sheehan *et al.*, 1999)

| 1940's | 1950's | 1960's | 1970's | 1980's | 1990's |
|---|---|---|---|---|---|
| 1944<br>First antifungal azole reported | 1956<br>Amphotericin B antifungal activity reported | 1960<br>Amphotericin B introduced | 1974<br>Econazole introduced | 1981<br>Ketoconazole oral formulation approved in US | 1990-92<br>First systemic triazoles fluconazole and itraconazole introduced in US |
| 1949<br>First polyene nystatin identified | 1958<br>First azole antifungal marketed | 1962<br>Flucytosine antifungal activity reported | 1979<br>Miconazole parenteral formulation introduced in UK | 1981<br>First allylamine natifine* in clinical trials | 1995-96<br>Second allylamine terbinafine* approved and amphotericin B lipid formulations approved |
| | | 1969<br>Miconazole and clotrimazole (topical) introduced | | 1987<br>Polyene lipid formulations in development | 1997<br>Itraconazole oral solution approved |

* Trials are under way. Not discussed in text.

reported in isolates from cancer patients with prolonged neutropenia (Lambert and O'Grady, 1992).

### (a) Amphotericin B

This agent acts by inhibiting fungi through an interaction with ergosterol (a fungal membrane sterol). This effect results in the loss of membrane-selective permeability and intracellular components, which in turn causes impairment of barrier functions, leakage of cellular components, and cell death. Depending on the concentration used, amphotericin B will exert either a fungistatic or a fungicidal effect. At low concentrations, leakage of cell constituents is restricted to small molecules and ions (sodium and potassium), and the damage is reparable. However, a paradoxical stimulatory effect of amphotericin B, which results in an increase in the number of colony-forming units of C. *albicans*, has also been reported (Brajtburg *et al.*, 1981). At high concentrations of the drug, larger molecules are transported through the membrane, producing irreversible loss of cell constituents and its subsequent disruption. Since mammalian cell membrane also contains sterols, this drug accounts for a certain degree of host toxicity. However, the polyenes bind more effectively to ergosterol, the principal sterol in fungal membranes, than to other mammalian sterols such as cholesterol (Warnock, 1991; Lambert and O'Grady, 1992; Finch and Snyder, 1994; Lesse, 1995). Its spectrum of activity includes most fungi that cause disease in man, including *Candida* spp.

Amphotericin B is poorly absorbed from the intestinal tract and is usually administered intravenously or topically. After an intravenous injection, the drug is rapidly sequestered in tissues and then is slowly released, with an initial half-life of 1-2 days followed by a slow elimination phase of about 15 days. It is highly protein-bound in serum, and this characteristic may partly account for its poor penetration into many body sites, including the CSF, the urine, and the chambers of the eye. The major route of its excretion is extra-renal (Lambert and O'Grady, 1992; Finch and Snyder, 1994).

Since this antimycotic is a broad-spectrum agent that is active against yeast and yeast-like as well as dimorphic and filamentous fungi, it is the drug of choice in most systemic mycoses. However, the duration of treatment will depend upon the severity of fungal infection as well as the intensity of underlying illness, as in the case of HIV infection.

Though not very popular, topical amphotericin B oral preparations (lozenges, mouthwashes, creams, ointments) are available for the treatment of oral candidosis. While topical therapy may be useful in its own right in primary oral candidosis, it could be used as an adjunct to parenteral therapy in secondary candidosis, which manifests both systemically as well as on mucosal surfaces.

Apart from the fungicidal effect, amphotericin B is capable of suppressing many pathogenic attributes of C.

**TABLE 3**

**Antifungal Agents Used in the Treatment of Oral Candidoses** (from various sources)

| Drug | Form | Dosage | Comments |
|---|---|---|---|
| Amphotericin B | Lozenge, 10 mg | Slowly dissolved in mouth 3-4 X/day after meals for 2 wks minimum. | Negligible absorption from gastrointestinal tract. When given IV for deep mycoses may cause thrombophlebitis, anorexia, nausea, vomiting, fever, headache, weight loss, anemia, hypokalemia, nephrotoxicity, hypotension, arrhythmias, etc. |
| | Oral suspension, 100 mg/mL | Placed in the mouth after food and retained near lesions 4X/day for 2 wks. | |
| Nystatin | Cream | Apply to affected area 3-4 X/day. | Negligible absorption from gastrointestinal tract. Nausea and vomiting with high doses. |
| | Pastille, 100,000 units | Dissolve 1 pastille slowly after meals 4X/day, usually for 7 days. | |
| | Oral suspension, 100,000 units | Apply after meals 4X/day, usually for 7 days, and continue use for several days after post-clinical healing. | |
| Clotrimazole | Cream | Apply to the affected area 2-3 times daily for 3-4 wks. | Mild local effects. Also has anti-staphylococcal activity. |
| | Solution | 5 mL 3-4 times daily for 2 wks minimum. | |
| Miconazole | Oral gel | Apply to the affected area 3-4 times daily. | Occasional mild local reactions. Also has antibacterial activity. Theoretically the best antifungal to treat angular cheilitis. Interacts with anticoagulants (warfarin), terfenadine, cisapride, and astemizole. Avoid in pregnancy and porphyria. |
| | Cream | Apply twice *per* day and continue for 10-14 days after the lesion heals. | |
| Ketoconazole | Tablets | 200-400-mg tablets taken once or twice daily with food for 2 wks. | May cause nausea, vomiting, rashes, pruritus, and liver damage. Interacts with anticoagulants, terfenadine, cisapride, and astemizole. Contraindicated in pregnancy and liver disease. |
| Fluconazole | Capsules | 50-100-mg capsule once daily for 2-3 wks. | Interacts with anticoagulants, terfenadine, cisapride, and astemizole. Contraindicated in pregnancy, liver and renal disease. May cause nausea, diarrhea, headache, rash, liver dysfunction. |
| Itraconazole | Capsules | 100-mg capsules daily taken immediately after meals for 2 wks. | Interacts with terfenadine, cisapride, and astemizole. Contraindicated in pregnancy and liver disease. May cause nausea, neuropathy, rash. |

*albicans* at lower concentrations. For instance, it suppresses adhesion of C. *albicans* to buccal epithelial cells (Brenciaglia *et al.*, 1986; Macura, 1988; Vuddhakul *et al.*, 1988; Abu-El Teen *et al.*, 1989), and inhibits their germ tube formation (Brenciaglia *et al.*, 1986; Abu-El Teen *et al.*, 1989). On the other hand, amphotericin B pre-exposed denture acrylic

suppresses candidal adhesion to a significant extent (McCourtie *et al.*, 1986). The former effects may be due to the mechanism of action of amphotericin B on the candidal cell wall, while the latter may be due to the blocking of the yeast attachment sites on the denture acrylic by the drug.

*Candida* is also well-known to produce enzymes, such as secretory aspartyl proteinases (Saps), which facilitate both attachment to and penetration of the mucous membranes (Samaranayake and MacFarlane, 1990). Interestingly, subminimal inhibitory concentrations (sub-MIC) of amphotericin B are capable of curtailing the activity of this enzyme in oral C. *albicans* isolates (Wu *et al.*, 1996), thus reducing its pathogenicity. On the contrary, exposure of C. *albicans* to sub-MIC of amphotericin B has resulted in enhanced resistance to apo-lactoferrin-mediated candidal cell death (Nikawa *et al.*, 1994). Apo-lactoferrin is an important antifungal proteinaceous component of saliva. Recent literature therefore indicates that amphotericin B has a multiplicity of effects on *Candida*, killing the organism at high concentrations and adversely modulating the pathogenic attributes of the yeast at lower concentrations. The effects of sub-therapeutic concentrations of antifungal agents, including the post-antifungal effect (PAFE), will be discussed later.

The most common and most serious adverse effect of systemic amphotericin B is nephrotoxicity (Table 3). Hypokalemia and mild anemia are also common. Other rare adverse effects include acute hypersensitivity reactions such as anaphylaxis, fever, and headache; vomiting, anorexia, backache, seizures, and thrombophlebitis at the site of injection have also been reported (Lambert and O'Grady, 1992; Finch and Snyder, 1994). Amphotericin B may potentiate nephrotoxicity of other agents such as aminoglycosides and cyclosporin, while concomitant administration of glucocorticoids may exacerbate electrolyte disturbances, especially hypokalemia. Synergistic and antagonistic effects have been reported with other antifungal agents and amphotericin B. Mechlorethamine and other anticancer agents may potentiate the nephrotoxic and hypotensive effects of amphotericin B (Lesse, 1995).

The currently available preparations for oral delivery of amphotericin B include an ointment, suspensions, creams, and lozenges. Inhibitory concentrations of amphotericin B can be detected in saliva up to 2 hrs after a single lozenge dose (Samaranayake and Ferguson, 1994). The oral dose for adults is 100-200 mg every 6 hrs. Lozenges (10 mg) can be given every 8 hrs to a maximum of 80 mg/day. Also, 1 mL of oral suspension (100 mg/mL) taken after food and retained near the lesion every 8 hrs is recommended. Intravenous infusions can be administered, with care, to adults and children at 0.25 mg/kg daily, with a maximum of 1.5 mg/kg daily in severely ill patients (Lambert and O'Grady, 1992).

**(b) Nystatin**

Nystatin is a polyene antifungal which has a mode of action identical to that of amphotericin B. It too prevents the biosynthesis of ergosterol in the fungal cell membrane, which is important for the fluidity and integrity of the membrane and for the function of many membrane-bound enzymes, including chitin synthetase, which is necessary for proper cellular growth and division (White *et al.*, 1998). Blocking of ergosterol biosynthesis alters the permeability of the yeast cell membrane, resulting in leakage of cell constituents and death. Nystatin is probably the most popular agent for treating superficial fungal infections caused by C. *albicans*. It has both a fungicidal and a fungistatic activity, depending on the concentration administered.

Due to its systemic toxicity, nystatin is used topically in the treatment of mucocutaneous infections caused by C. *albicans*. Pharmaceutical preparations of nystatin contain a heterogenous mixture of compounds in addition to the main ingredient, and hence the biological activity of nystatin is commonly expressed in international units (IU) (Samaranayake and Ferguson, 1994).

Nystatin is available in creams, tablets, suspensions, oral rinses, gels, and pastilles (Lesse, 1995). The ointment contains perfumes and other agents and is not suitable for intra-oral use, but has been used for the treatment of angular cheilitis. Nystatin tablets (500,000 IU) are commonly used for the treatment of oral candidosis, as are unflavored vaginal tablets (100,000 IU). The latter is highly efficacious when used orally as long as the patient is persuaded to take them; the bitter taste of the tablets, however, results in poor patient compliance (Greenspan, 1994). The suspension can be used for young children or in patients where there is poor compliance, although its rapid clearance from the oral cavity results in concentrations falling to sub-therapeutic levels fairly quickly. Similarly, the oral rinse is relatively ineffective, because of the short contact time with the oral mucosa. Further, it contains sucrose and increases the risk of dental caries (Greenspan, 1994). In contrast, the pastilles and lozenges can be sucked slowly and hence have a longer duration of action. Further, the sweetened formulations of pastilles and lozenges result in better patient compliance, and, due to their prolonged retention, pastilles can be expected to be a better fungicidal agent than the suspension (Millns and Martin, 1996). Nystatin pastilles are ideal for the treatment of *Candida*-associated denture stomatitis (Martin *et al.*, 1986), and could be used to prevent outbreaks or recurrence of oral candidosis in HIV-infected patients (MacPhail *et al.*, 1996). However, since these are also sweetened with sucrose, it will increase the risk of causing dental caries and may be contraindicated in dentate, caries-prone individuals.

Some studies have shown that a slow-release system with nystatin provides rapid clinical and mycological improvement in patients with oral candidosis. This slow-release form, kept in the mouth and not swallowed, is sugar-free and offers a prolonged contact time. It has been more effective than the pastilles for up to a week after treatment. Nystatin has also been incorporated into a controlled drug delivery system and marketed as a mucosal oral therapeutic system (MOTS). In a study of HIV-infected patients with oral candidosis, the MOTS was shown to be more effective than the oral nystatin pastilles in lesion resolution (Samaranayake and Ferguson, 1994). Although the MOTS appears to be a significant step forward in the oral delivery of nystatin, further studies are warranted prior to its general release.

Nystatin has been studied for its effectiveness for eradicating yeasts from denture surfaces. In a study where the effectiveness of a denture-soaking nystatin solution was evaluated for the treatment of oral candidosis in old, chronically ill, institutionalized adults, the outcome was not satisfactory (Banting *et al.*, 1995). Although the clinical signs and symptoms of oral candidosis were resolved in all these subjects following therapy, the presence of invasive candidal hyphae was detected in approximately 80% of tissue and/or dentures. Since there are more appropriate disinfectant agents such as hypochlorite and chlorhexidine gluconate for the overnight disinfection of dentures, the use of nystatin for this purpose is questionable and cannot be condoned.

The effects of low concentrations of nystatin on the pathogenic attributes of *Candida*, such as their adhesion to host surfaces and their proteolytic activity, are not widely known. Nystatin, like amphotericin B, is capable of suppressing adhesion of C. *albicans* to buccal epithelial cells both *in vivo* and *in vitro* (Macura, 1988; Vuddhakul *et al.*, 1988; Abu-El Teen *et al.*, 1989; Darwazeh *et al.*, 1997), and also to vaginal epithelial cells (Braga *et al.*, 1996). Further, nystatin was shown to be capable of reducing oropharyngeal colonization by C. *albicans* in CD4+ T-cell-deficient mice (Flattery *et al.*, 1996), and reducing candidal adhesion to denture acrylic pre-treated with the drug (McCourtie *et al.*, 1986). In addition, the ability of nystatin to cause inhibition of germ tube formation by C. *albicans* has also been reported (Abu-El Teen *et al.*, 1989). Since nystatin acts on the candidal cell wall, the foregoing effects, which adversely modulate the pathogenic attributes related to the outer surface of the yeast, are not surprising.

The proteolytic activity of oral C. *albicans* isolates *in vitro* is also curtailed by nystatin (Wu *et al.*, 1996). However, similar to amphotericin B, exposure of C. *albicans* to sub-MIC of nystatin has resulted in an increased resistance to apo-lactoferrin-mediated cell death (Nikawa *et al.*, 1994).

Several topical preparations of nystatin can be used in the treatment of oral candidosis. These include: dissolved vaginal tablets (100,000 IU), 1 tablet 3 times a day; dissolved pastille (100,000 IU), 1-2 pastilles 3-4 times a day; ointment/cream to be applied to commissures 3 times a day; and oral suspension (100,000 units/mL) 4 times a day, continued for several days after post-clinical healing (Table 3; Greenspan, 1994).

#### (c) Natamycin

Natamycin is active against *Candida* when applied topically but has a broader range of activity against fungi than do the other two polyenes. For oral use, it is prepared as a 1% suspension, but ointment and tablets are also available (Samaranayake and Ferguson, 1994). However, this drug is not widely used.

### (B) Azole antifungal agents

These agents are classified into two groups: (1) the imidazoles—clotrimazole, econazole, fenticonazole, isoconazole, ketoconazole, miconazole, sulconazole, and tioconazole; and (2) the triazoles—fluconazole and itraconazole.

The azoles are becoming increasingly popular in the management of oral candidosis (Greenspan, 1994; Samaranayake and Ferguson, 1994). Indeed, fluconazole is the drug of choice in the treatment of oro-pharyngeal candidosis in HIV infection. The azole antifungals act by inhibition of cytochrome p-450 enzyme that is involved in cell membrane synthesis in fungi. The principal target is 14α-demethylase, which converts 14α-methylsterols to ergosterol in the fungal cell membrane. Therefore, they cause alteration of the fungal cell membrane by blocking the 14α-demethylation step in the synthesis of ergosterol (depletion of ergosterol and accumulation of 14α methylsterols), an important constituent of fungal cell membrane, which thus becomes permeable to intracellular constituents and leads to alterations in several membrane-associated functions. Imidazoles, in addition, interfere with fungal oxidative enzymes to cause lethal accumulation of hydrogen peroxide. The selective toxicity of azoles is due to their differential affinities for mammalian and fungal cytochrome p-450 (Lambert and O'Grady, 1992; Finch and Snyder, 1994; Lesse, 1995).

Clotrimazole, econazole, fenticonazole, isoconazole, miconazole, sulconazole, and tioconazole are used for local treatment. However, miconazole can also be given by mouth for oral and intestinal infections. Ketoconazole, fluconazole, and itraconazole are used for both local and systemic candidoses.

#### (a) Clotrimazole

Clotrimazole has a broad spectrum of activity, being anti-candidal as well as anti-staphylococcal, and is primarily

fungistatic (Table 3; Samaranayake and Ferguson, 1994). It is mainly used in the management of superficial candidal infections in the oral cavity, skin, and vagina. Topical application results in adequate therapeutic concentrations in the epidermis or the mucosa. It is particularly effective in managing oropharyngeal candidosis, especially in immunocompromised, such as HIV-infected, patients (Greenspan, 1994), and recipients of renal transplants (Gombert *et al.*, 1987). It is widely used as a first-line treatment of oral candidosis, especially in the USA. As a cream, it is particularly useful in the treatment of angular cheilitis, due to its dual action on both yeasts and staphylococci (Greenspan, 1994).

Clotrimazole is known to suppress candidal adhesion to human buccal epithelial cells, a factor which may aid its therapeutic efficacy (Macura, 1988). Further, sub-inhibitory concentrations of clotrimazole have been shown to curtail the proteinase production by oral C. *albicans* isolated from HIV-positive and HIV-negative individuals (Wu *et al.*, 1996). In contrast, clotrimazole had no effect on the adherence of C. *albicans* to vaginal epithelial cells, *in vitro*, regardless of whether the drug was used to pre-treat the fungi or the vaginal epithelial cells or was added to the yeast/vaginal cell mixture (Odds and Webster, 1988).

When applied topically, clotrimazole is well-tolerated. Adverse reactions are minor and rare, and include local skin irritation, vomiting, and nausea. Abdominal cramps, increased urination, and elevated liver SGOT levels have also been reported (Finch and Snyder, 1994).

Clotrimazole is available in the form of 1% cream which can be applied to commissures three times a day, and oral troche (10 mg) to be used dissolved 5 times a day. Other forms—such as lozenges, vaginal creams, and vaginal tablets—are also available for topical use (Lesse, 1995). The most common method of delivery of clotrimazole in oral candidosis is the use of a troche, available in 10-mg units. Slow dissolution in the mouth is thought to result in binding of clotrimazole to the oral mucosa, which is then gradually released to maintain a fungistatic concentration for several hours. Patient compliance is said to be enhanced by the more pleasant taste of clotrimazole as compared with the bitter flavor of certain nystatin preparations, such as tablets and vaginal pessaries, which are specifically not formulated for oral use (Samaranayake and Ferguson, 1994).

### (b) Miconazole

Miconazole, like clotrimazole, has a broad spectrum of activity against fungi, including C. *albicans*. It is also effective against some Gram-positive bacteria, such as staphylococci, and hence is useful in the management of angular cheilitis where concurrent bacterial and fungal infection may be present (Martin, 1990).

Miconazole can be given either topically, intravenously, or intrathecally. Only small amounts are absorbed through the skin or from vaginal mucous membrane. Serum concentrations as high as 7.5 mg/L, 15 min after an infusion of a large dose of 100 mg, have been reported (Lambert and O'Grady, 1992). Initial half-life of the drug is about 20 to 30 min, but following continuous drug administration, it is increased to about 24 hrs. Miconazole is rapidly metabolized in the liver. Excretion is essentially non-renal and is eliminated by hepatic metabolism, mainly in the bile. There is good penetration of the drug into inflamed joints, eyes, and the peritoneal cavity. Penetration into saliva, sputum, and the CNS is poor (Finch and Snyder, 1994).

Miconazole is effective in all types of oral candidosis, including chronic mucocutaneous candidosis. It is also used in vulvovaginitis caused by C. *albicans*. The systemic use of miconazole has been largely superseded by the availability of other, less toxic, drugs such as ketoconazole and fluconazole (Finch and Snyder, 1994).

Low concentrations of miconazole are capable of suppressing candidal adhesion to buccal epithelial cells (Brenciaglia *et al.*, 1986; Vuddhakul *et al.*, 1988; Abu-El Teen *et al.*, 1989) and inhibiting germ tube formation in C. *albicans* (Borgers *et al.*, 1979; Johnson *et al.*, 1983; Abu-El Teen *et al.*, 1989). In contrast, such inhibitory effect on germ tubes was not seen in another study (Brenciaglia *et al.*, 1986). Interestingly, as is the case with clotrimazole, proteinase production by oral C. *albicans* was also curtailed by sub-MIC of miconazole (Wu *et al.*, 1996).

Side-effects after the topical use of miconazole are few and uncommon. Burning and skin maceration can occur following cutaneous use. Itching, burning, urticaria, headache, and cramps have been associated with the use of vaginal preparations. The most common side-effect after intravenous use is thrombophlebitis. Nausea may develop in some cases. Rarely, anaphylaxis and cardiotoxicity can occur (Finch and Snyder, 1994).

Oral anticoagulants such as warfarin are widely prescribed in the management of deep vein thrombosis, pulmonary embolism, and patients with prosthetic heart valves. Systemic antifungal drugs of the azole group are known to enhance the anticoagulant effect of warfarin (Baciewicz *et al.*, 1994). Recently, it has been reported that the concurrent use of miconazole oral gel for the treatment of oral candidosis resulted in potentially life-threatening derangement of warfarin anticoagulation (Table 3; Ariyaratnam *et al.*, 1997; Ezsias *et al.*, 1997; Pemberton *et al.*, 1998).

Tablets, oral gel, intravenous injections, and topical and vaginal preparations are available for the treatment of candidosis. Miconazole cream is a very effective delivery mode for angular cheilitis lesions caused by *Candida* and *Staphylococcus aureus* (Table 3). Another advantage is

that the drug can be administered empirically when a microbial report is not available or when the facility to identify the exact nature of the infective agent is inaccessible (Samaranayake and Ferguson, 1994). Miconazole has been formulated into a lacquer and is effective in the treatment of *Candida*-associated denture stomatitis. At least two studies have reported that a single application of a miconazole lacquer painted over the fitting surface of the denture, as a slow delivery agent, considerably reduces the numbers of *Candida* on the denture surfaces for a substantial period of time, and leads to a clinical resolution of the mucosal lesion (Konsberg and Axéll, 1994; Dias *et al.*, 1997).

**(c) Ketoconazole**

Ketoconazole is effective against a wide spectrum of fungi and yeasts, including *Candida* spp., but, unlike other azoles such as miconazole, it has no antibacterial activity (Martin, 1990). It has been used in the management of cutaneous, oral, esophageal, and vaginal *Candida* infections for several years (Lesse, 1995). Ketoconazole has no place in the treatment of primary oral candidoses, and its main indication is for secondary oral candidoses such as in chronic mucocutaneous candidosis (Samaranayake and Ferguson, 1994). However, triazoles such as fluconazole are being increasingly used for the latter diseases.

Mucosal candidoses of the mouth and esophagus respond well to ketoconazole, although there is little evidence to suggest that the latter is better than nystatin in granulocytopenic patients (Finch and Snyder, 1994). On the contrary, ketoconazole has been shown to be superior to nystatin in reducing oropharyngeal candidal colonization in mice depleted of CD4+ lymphocytes (Flattery *et al.*, 1996). However, when 85 HIV-positive patients with oral candidosis were evaluated for response to systemic antifungal treatment with ketoconazole, 65 responded with complete clinical remission to 200 mg daily, after seven days of ketoconazole treatment, even though 81% of post-treatment cultures remained positive (Silverman *et al.*, 1996).

Unlike other imidazoles, ketoconazole is readily absorbed after oral administration, especially at an acidic pH. However, absorption is variable, with peak plasma levels of approximately 3.5 mg/mL achieved 1-2 hrs after administration of 200 mg. The passage of ketoconazole into the cerebro-spinal fluid is generally poor and unreliable, since greater than 90% is bound to serum protein during bodily distribution. It is metabolized in the liver, and inactive metabolites are excreted, largely in bile and, to a smaller extent, in urine (Lambert and O'Grady, 1992; Finch and Snyder, 1994).

Though the mechanisms by which ketoconazole reduces mucosal colonization are not fully understood a marked reduction of germination and adhesion of C. *albicans* to vaginal and buccal epithelial cells following exposure to ketoconazole has been reported (Johnson *et al.*, 1983; Sobel and Obedeanu, 1983; Macura, 1988; Vuddhakul *et al.*, 1988; Abu-El Teen *et al.*, 1989). In contrast, one group has reported that the adhesion of C. *albicans* to vaginal epithelial cells, *in vitro*, was not significantly altered when the drug was used to pre-treat the fungi or the vaginal cells or was added to the fungal/vaginal cell mixture (Odds and Webster, 1988).

The most common adverse reactions to ketoconazole are gastro-intestinal intolerance, with nausea and vomiting. Hepatotoxicity is not uncommon but is generally asymptomatic and accompanied by a reversible elevation of serum transaminase (Table 3). Care has to be taken during administration of ketoconazole, since fatal hepatotoxicity and nephrotoxicity have been reported. Liver function tests should be performed throughout any prolonged ketoconazole therapy, and treatment should be discontinued in patients with progressively increasing transaminase levels (Odds, 1994). In subclinical adrenocorticosteroid deficiency, ketoconazole blocks steroid synthesis in host cells. Depression of testosterone biosynthesis can manifest as painful gynacomastia, loss of libido, and sometimes loss of hair (Lambert and O'Grady,1992). It is also a potential teratogenic agent (Fromtling, 1984). Because of the possible effects on the liver and steroid metabolism, it should not be used as a first-line treatment for mucosal or cutaneous infections. But if used carefully, it can be very effective, and the risk and side-effects relatively minor (Martin, 1990).

Many drug interactions are seen with ketoconazole. Some are mild; others may have life-threatening consequences. Ketoconazole is capable of decreasing the hepatic metabolism of non-sedative antihistamines such as terfenadine and astemizole, which can lead to increased levels of the latter and its metabolites, with resultant arrhythmias, tachycardia, and, rarely, death (Lesse, 1995). Similarly, ketoconazole can suppress the metabolism of cyclosporine, leading to elevated concentrations and accompanying profound immunosuppression and renal dysfunction, which may be life-threatening. Absorption of ketoconazole may be reduced by antacids and $H_2$-receptor blockers such as cimetidine and ranitidine. Rifampin, a potent inducer of hepatic metabolizing enzymes, can decrease ketoconazole concentration in serum (Lesse, 1995).

Tablets, suspensions, and creams of ketoconazole are available. Depending on the infection, a 200- to 400-mg tablet once daily is the dosage for systemic use (Lambert and O'Grady, 1992; Greenspan, 1994). A 2% cream can be successfully applied to commissures three times a day in chronic hyperplastic candidosis.

As opposed to the imidazole antifungals discussed

above, itraconazole and fluconazole are triazoles, which differ somewhat in their pharmacological effects. Itraconazole is water-insoluble and lipophilic, and requires a lower pH to be ionized, as is true of ketoconazole; it is highly protein-bound and is excreted in bile. Fluconazole, on the other hand, is water-soluble and does not require a lower gastric pH for absorption; it is poorly bound to plasma protein and is eliminated by renal excretion. An important aspect of fluconazole is that it reaches high concentrations in the normal and inflamed CNS (Finch and Snyder, 1994). A major therapeutic advantage of triazoles is that they are more specific than the imidazoles for the fungal rather than the mammalian cytochrome p-450 enzyme (Lesse, 1995), with resultant lower toxicity and fewer side-effects.

**(d) Fluconazole**

Fluconazole has a broad spectrum of antifungal activity. It is active against most strains of C. *albicans* but is less active against non-*albicans Candida* species, particularly C. *krusei* and C. *glabrata*, which are intrinsically resistant to the drug (Van't Wout, 1996; Samaranayake, 1997; White *et al.*, 1998). *In vitro* sensitivity of this drug is highly dependent on the test conditions. Thus, it is not unusual to obtain very high MIC values, *in vitro*, for strains which are responsive to the drug *in vivo* (Minguez *et al.*, 1994). Further, it has been shown that although fluconazole is remarkably more effective than ketoconazole in the treatment of candidal infections (Martin, 1989), its growth-inhibitory activity against C. *albicans*, *in vitro*, is considerably less than that of ketoconazole (Troke *et al.*, 1988). Interestingly, it has been shown that fluconazole 400 mg daily is effective against oropharyngeal and esophageal candidosis in a patient with endocrinopathy syndrome, despite the infecting C. *albicans* strains being resistant to azole antifungals *in vitro* (Field *et al.*, 1996).

Fluconazole is given either orally or intravenously. It is well-absorbed after oral administration, and peak serum concentrations are reached within 2-4 hrs. What distinguishes fluconazole from many other azoles is this excellent absorption from the gastro-intestinal tract, coupled with a very long serum half-life. The half-life in adults ranges between 27 and 37 hrs. It also differs from other azole antifungals in being weakly protein-bound in serum. This helps in its excellent passage into most body sites (Lambert and O'Grady, 1992). Unlike other azoles, fluconazole is not metabolized in man, and approximately 80% is excreted, largely through the kidney, unchanged (Finch and Snyder, 1994). It follows, therefore, that fluconazole has an almost negligible effect on hepatic function as compared with other azoles, which are metabolized mostly in the liver, leading to possible hepatotoxicity.

Though fluconazole was introduced in late 1980's, there is a considerable amount of data based on its efficacy in the management of mucosal/oral candidoses. Some of this information is given below. The high systemic absorption of fluconazole has been useful in treating oral candidosis in HIV-infected patients, and it is now considered the drug of choice for candidoses in HIV disease (Dupont and Drouhet, 1988; Lucatorto *et al.*, 1991). It has been shown that weekly fluconazole (200 mg) is safe and effective in preventing oropharyngeal and vaginal candidosis, and this regimen has a useful role in the management of HIV-infected patients who are at risk for recurrent mucosal candidosis (Schuman *et al.*, 1997). Further, when human immunodeficiency virus (HIV)-infected patients with oropharyngeal candidosis were randomly assigned to receive 14 days of therapy with a liquid suspension of fluconazole (100 mg daily) or liquid nystatin (50,000 IU, four times daily), it was found that fluconazole oral suspension as a systemic therapy was more effective than liquid nystatin as a topical therapy in the treatment of oral candidosis, and provided a longer disease-free interval before relapse (Pons *et al.*, 1997). When the effectiveness of antifungal treatment for thrush in HIV-infected patients was observed, it was shown that both fluconazole and clotrimazole were equally effective in treating thrush, but mycological cure occurred more often with fluconazole (Sangeorzan *et al.*, 1994). Further, administration of fluconazole in capsule form has proved effective in the prophylaxis and treatment of mucosal candidosis in immunocompromised patients, and, in addition, a topical effect could be obtained in oro-pharyngeal candidosis with a fluconazole suspension (Wildfeuer *et al.*, 1996), possibly due its oral delivery *via* gingival crevicular fluid and saliva. For instance, it has been shown that, after a single dose, fluconazole (100 mg) achieved higher salivary concentrations than did ketoconazole (400 mg). This may explain the increased clinical efficacy of fluconazole in the treatment of oropharyngeal candidosis (Force and Nahata, 1995).

Fluconazole has also been shown to be effective in resolving palatal candidosis at a dose nine times lower than that of ketoconazole (Martin, 1989). It has been successfully used in the management of candidal leukoplakia (Lamey *et al.*, 1989), cheilo candidosis [a rare candidal infection of the lips due to associated intra-oral candidal carriage, actinic lip damage, and Sjögren's syndrome (Napier *et al.*, 1996)], and oral candidosis in bone marrow transplant patients (Goodman *et al.*, 1992), in malignant disease (Ninane *et al.*, 1994; Lesse, 1995), and in acute leukemia (Winston *et al.*, 1993). In recent studies, fluconazole (100 mg/daily) was compared with clotrimazole (10 mg 5 times a day) in patients with oral candidosis. At the end of two weeks, fluconazole was more effective in clearing *Candida*, and those treated with fluconazole remained free of infection for a longer

period than those treated with clotrimazole (Hay, 1990; Koletar *et al.*, 1990; Pons *et al.*, 1993). In patients with *Candida*-associated denture stomatitis, fluconazole is effective (Budtz-Jørgensen, 1990; Bissell *et al.*, 1993), especially when administered along with an oral antiseptic such as chlorhexidine (Hay, 1990; Kulak *et al.*, 1994; Arikan *et al.*, 1995).

Adhesion of *Candida* to epithelial cells, a major determinant of candidal colonization, is significantly inhibited by fluconazole (Darwazeh *et al.*, 1991). Since fluconazole is secreted in high concentrations in saliva (Laufen *et al.*, 1995; Wildfeuer *et al.*, 1996), it may interfere with the synthesis or structure of *Candida* receptors on buccal epithelial cells, which may help reduce candidal colonization. Further experimental evidence suggests that subinhibitory concentrations of fluconazole are capable of reducing the adhesiveness of *Candida* to vaginal mucosal cells, though to a lesser extent than nystatin (Braga *et al.*, 1996). On the contrary, fluconazole showed superior efficacy in reducing oral colonization by C. *albicans* in mice depleted of CD4+ T-lymphocytes (Flattery *et al.*, 1996). Further, systemic fluconazole therapy significantly inhibited the adherence of C. *albicans* to buccal epithelial cells of oral candidosis patients, and also interfered with the synthesis of candidal receptor-like proteins on the surfaces of buccal epithelial cells (Shaoxi *et al.*, 1996).

Fluconazole is well-tolerated, and side-effects such as nausea, headache, and gastro-intestinal and abdominal discomfort are usually mild and subjective (Table 3). It may cause elevation of liver enzymes and an allergic rash. Jaundice and abnormal liver function tests were seen in some patients treated with fluconazole in HIV-related oral candidal infection (Franklin *et al.*, 1990). It is also reported that a patient with AIDS-related oropharyngeal candidosis treated with fluconazole developed a dose-related liver dysfunction (Wells and Lever, 1992). Since animal studies have demonstrated embryotoxicity, it is prudent for fluconazole to be avoided in pregnancy (Aleck and Bartley, 1997; Laurence *et al.*, 1997).

Although fluconazole has less activity on mammalian cytochrome P-450 enzymes than does ketoconazole, and although the drug interactions are fewer, fluconazole nevertheless has significant interactions with several medications. Despite the paucity of reports of interactions with terfenadine and astemizole (non-sedative antihistamines), the structural analogy of fluconazole and ketoconazole indicates that non-sedating antihistamines should not be administered with fluconazole. By decreasing the hepatic metabolism of several agents, fluconazole can bring about high serum concentrations of such agents when administered concurrently. For instance, decreased clearance of cyclosporine may result in significant immunosuppression, leukopenia, and renal dysfunction, and a similar interaction with phenytoin, warfarin, and hypoglycemics can produce toxic phenytoin concentrations in serum, prolonged prothrombin times, and hypoglycemia, respectively (Baciewicz *et al.*, 1994; Lesse, 1995).

Fluconazole is available in capsule and intravenous formulations. For adults, the oral and intravenous dosage is 50 mg daily for 7-14 days for the treatment of oropharyngeal candidosis, while 50 mg daily for 14-30 days is recommended for the treatment of esophageal candidosis (Table 3; Lambert and O'Grady, 1992).

### (e) Itraconazole

Itraconazole is a lipophilic drug that is well-absorbed after oral administration and has a wide spectrum of antifungal activity. It is effective in various superficial mycoses, including oral candidosis due to C. *albicans* as well as C. *krusei* and C. *glabrata* (Van Cutsem, 1994). Following drug administration, peak serum concentrations are reached 2-4 hrs later, and greater than 99% is bound to plasma proteins. It requires a low gastric pH for absorption. In lipophilic tissues, the concentration of the drug is 2 to 20 times higher than the plasma concentration. It is metabolized in the liver; excretion is biliary (Lambert and O'Grady, 1992; Finch and Snyder, 1994).

Since C. *krusei* and C. *glabrata* are intrinsically resistant to fluconazole (Samaranayake, 1997), itraconazole is an ideal alternative in the management of patients infected with fluconazole-resistant *Candida* (Ruhnke *et al.*, 1994; Phillips *et al.*, 1996; Laguna *et al.*, 1997). For instance, when 40 AIDS patients suffering from fluconazole-resistant oro-pharyngeal candidosis were treated with oral itraconazole solution (200-800 mg *per* day), the condition was significantly improved (Eichel *et al.*, 1996). In the treatment of oral candidosis, one study has shown that patients treated with itraconazole (200 mg/day) had a longer period of remission than did patients treated with ketoconazole (Smith *et al.*, 1988). In two separate comparative studies, itraconazole produced a faster response rate and a longer time before relapse than did clotrimazole (Blatchford, 1990).

Generally, itraconazole is well-tolerated, though gastro-intestinal disturbances, headache, and dizziness have been reported (Finch and Snyder, 1994). Transient, asymptomatic transaminase elevations and hypokalemia have also been reported (Lambert and O'Grady, 1992). As with other azoles, cyclosporine clearance is reduced by itraconazole, and serum concentrations of the former should be monitored to prevent the occurrence of potentially major complications. Similarly, simultaneous use of itraconazole and either terfenadine or astemizole should be avoided. Itraconazole has been reported to decrease digoxin clearance, and serum digoxin concentrations should be measured during con-

current therapy (Lesse, 1995).

Itraconazole is available in capsule and oral solution forms. The adult oral dose is 100 mg daily for 15 days for oro-pharyngeal candidosis (Table 3; Lambert and O'Grady, 1992). The availability of an oral solution may be advantageous over the capsule formulation, due to the fact that it would be easier to swallow for patients with severe oro-pharyngeal candidosis (Blatchford, 1990).

### (f) Drawbacks of azoles

All azoles are fungistatic, not fungicidal. This is an important consideration in the treatment of chronic, immunocompromised patients, such as those with AIDS, and in the treatment of infections at critical sites (*e.g.*, candidal meningitis) (Siegman-Igra and Raban, 1992). Further, none of the azoles is entirely benign, and they are expensive. Hepatotoxicity may be common to all of them (Lesse, 1995), and the potential for endocrine toxicity exists, particularly at higher doses.

Although antifungal drug resistance has been extensively reviewed recently (White *et al.*, 1998), some points are worthy of note. The emergence of resistance to the triazoles, particularly to fluconazole, is disturbing. Fluconazole resistance has been defined as the persistence of clinical candidosis despite treatment with 100 mg daily for at least 7 days (Dupont *et al.*, 1996). It is now a real clinical problem in patients with HIV disease (Barchiesi *et al.*, 1995; Johnson *et al.*, 1995; Heald *et al.*, 1996; McGinnis and Rinaldi, 1996; Dromer *et al.*, 1997; Klepser *et al.*, 1997; White *et al.*, 1997). A recent study found that at least one fungus resistant to fluconazole was isolated from 41% of patients with AIDS (Maenza *et al.*, 1997). Fluconazole-resistant *Candida* is also a problem, especially in intravenous drug users (Chavanet *et al.*, 1994) and in those with systemic candidiasis (Siegman-Igra and Raban, 1992). It has also been reported in patients with chronic mucocutaneous candidosis (Field *et al.*, 1996).

There is also evidence of cross-resistance between fluconazole-resistant C. *albicans* and non-*albicans* isolates to ketoconazole, miconazole, and itraconazole (Le Guennec *et al.*, 1995; Maenza *et al.*, 1996). Nonetheless, other studies have indicated that ketoconazole and itraconazole may be clinically effective, despite some cross-resistance (Laguna *et al.*, 1997). Itraconazole, in particular, may remain effective (Martinez *et al.*, 1997), though about 30% of fluconazole-resistant isolates may still be resistant to itraconazole (Cartledge *et al.*, 1997). Fortunately, there may still be a clinical response to amphotericin B in fluconazole-resistant candidiasis (Maenza *et al.*, 1996; Revankar *et al.*, 1996).

Three main resistance patterns of fluconazole have been identified: first, a progressive increase in the MIC over weeks or months, and infection with the same *Candida* strain; second, a sudden or rapid failure of therapy, with the emergence of a new resistant strain; and finally the acquisition of a new and resistant organism from a sexual partner.

Fluconazole resistance appears to result from mutation (Barchiesi *et al.*, 1996) and may appear in patients who have received no fluconazole, since resistance may be transferred (Goff *et al.*, 1995; Revankar *et al.*, 1996). Thus, transfer of fluconazole-resistant *Candida* species between patients has been documented (Barchiesi *et al.*, 1995; Boerlin *et al.*, 1995), perhaps due to the patients' sexual activity (Dromer *et al.*, 1997). Further, previous fluconazole use and severe immune defects are risk factors for fluconazole resistance (Revankar *et al.*, 1996; Laguna *et al.*, 1997; Tumbarello *et al.*, 1997). There is also evidence that intermittent fluconazole or low-dose therapy is more likely to result in the emergence of resistance isolates than continuous and/or high-dose therapy (Chavanet *et al.*, 1994; Ruhnke *et al.*, 1994; Heald *et al.*, 1996). Azole resistance can also arise because of changes in the candidal target enzyme 14α sterol demethylase, reduced fungal membrane permeability to azoles, or increased efflux of azoles from the organisms (Sanglard *et al.*, 1995; Clark *et al.*, 1996; Lamb *et al.*, 1997; Marichal *et al.*, 1997).

Potential resistance to fluconazole may be predicted by rather tedious, *in vitro*, assays proposed by the US National Committee for Clinical Laboratory Standards (NCCLS). However, the commercially available "E test" could be used for this purpose, since it is simple and reliable (Dannaoui *et al.*, 1997). Others have proposed newer methods of susceptibility testing using flow cytometry (Ramani *et al.*, 1997).

### (g) Treatment of fluconazole-resistant candidosis

Management strategies for fluconazole-resistant cases include: (a) higher oral doses of fluconazole, up to 600 mg *per* day; (b) use of a fluconazole suspension as an oral rinse (Martins and Rex, 1997); and (c) the use of systemic ketoconazole 400 mg *per* day or itraconazole 200-400 mg *per* day (Ng and Denning, 1993). Several reports indicate that itraconazole is an effective alternative for fluconazole-resistant candidosis (Dupont *et al.*, 1996; Phillips *et al.*, 1996; Cartledge *et al.*, 1997). Amphotericin B used orally, intravenously, or liposomally may also be effective (Dewsnup and Stevens, 1994; Nguyen *et al.*, 1996).

## (C) DNA ANALOGUES

The drug 5-fluorocytosine is rarely, if ever, used in the management of oral candidoses. However, a brief overview is provided for the sake of clarity and comprehensiveness. The DNA analogue 5-fluorocytosine is a fungistatic agent which is highly effective against all

*Candida* spp. After administration, it is transported into the fungal cell by the action of cytosine permease, and inside the cell, is converted to form the active metabolite 5-fluorouracil by cytosine deaminase. 5-fluorouracil is then incorporated into RNA in place of uracil, with resulting abnormalities of protein synthesis. In addition, it blocks thymidylate synthetase, causing inhibition of DNA synthesis. Since the mammalian cells lack cytosine deaminase, they are not affected by the drug (Lambert and O'Grady, 1992; Finch and Snyder, 1994).

This drug is well-absorbed from the intestinal tract after oral administration, and the serum half-life of 5-fluorocytosine is between 3 and 6 hrs. Serum protein binding is minimal and is widely distributed, with CSF concentrations in the region of 75% of serum concentrations. 5-Fluorocytosine is mainly excreted through the renal route, thus necessitating modifications of the dosage regimen in renal dysfunction (Lambert and O'Grady, 1992).

Its principal use is in combination with amphotericin B in the treatment of deep forms of systemic candidosis, including fungal endocarditis. As C. *albicans* rapidly becomes resistant to 5-fluorocytosine, combination therapy with another antifungal, usually amphotericin B, is recommended. However, such combination increases the risk of adverse effects (Laurence *et al.*, 1997).

The most important toxic effect of 5-fluorocytosine is marrow aplasia, which can occur with long-term therapy and high serum concentrations. Vomiting, diarrhea and, rarely, more severe enteritis and hepatotoxicity can also occur.

Tablets and intravenous infusions are available for treatment, and the adult oral and intravenous dosage is 200 mg *per* kg body weight in four divided doses *per* day (Lambert and O'Grady, 1992).

## (D) Other antifungal agents used in oral candidosis

### (a) New antifungal agents

A vast array of new antifungal agents—such as amorolfine, natifine, terbinafine, tolaftate (McGinnis and Rinaldi, 1996), rilopirox (Braga *et al.*, 1996), cilofungin, pradimycin (Samaranayake and Ferguson, 1994), voriconazole (Ruhnke *et al.*, 1997), and benanomicin A (Ohtsuka *et al.*, 1997)—is either being marketed or undergoing extensive clinical trials. However, there are limited data on the oral pharmacodynamics of these agents, despite the fact that most are clinically very effective.

### (b) Chlorhexidine

Since its introduction in the 1970s, chlorhexidine has been used as an adjunct in the management of oral candidoses (Budtz-Jørgensen, 1990). For instance, 0.2% chlorhexidine gluconate has been successfully used as a mouthrinse in the treatment of *Candida*-associated denture stomatitis and in pseudomembranous candidosis, while 2% suspension is used as an overnight denture disinfectant.

Chlorhexidine gluconate has a bimodal action on *Candida*. First, it is fungicidal, even at very low concentrations, with MIC reaching 0.001% (Ellepola and Samaranayake, 1999a). Second, it is capable of significantly suppressing candidal adhesion to both inorganic and organic substrates. Many studies have clearly demonstrated the latter property of chlorhexidine gluconate, particularly in suppressing candidal adhesion to denture acrylic surfaces (McCourtie *et al.*, 1985, 1986; Kamalakshi *et al.*, 1992). Further, exposure of buccal epithelial cells to chlorhexidine gluconate, either *in vivo* or *in vitro*, significantly reduces the adhesion of *Candida* to buccal cells (Tobgi *et al.*, 1987; Audus *et al.*, 1992). Interestingly, this phenomenon persists regardless of whether the buccal cells are derived from healthy subjects or from patients with diabetes mellitus (Darwazeh *et al.*, 1994). Due to its multifaceted anti-candidal action, mouthrinses containing chlorhexidine have been proposed as an appropriate alternative to conventional antifungals in the management of oral candidosis (Giuliana *et al.*, 1997). It is salutary to note that chlorhexidine and nystatin should not be used simultaneously, since they interact, forming chlorhexidine-nystatin complexes, rendering both agents ineffective against *Candida* (Barkvoll and Attramadal, 1989).

### (c) Antifungal agents incorporated into dental materials

Due to the recalcitrant nature of *Candida*-associated denture stomatitis, several workers have attempted, with some degree of success, to incorporate antifungals such as nystatin into dental materials. For instance, nystatin has been incorporated into denture liners as an alternative for the treatment of denture stomatitis (Douglas and Walker, 1973). Further, miconazole and ketoconazole have been shown to be effective in suppressing candidal growth when combined with tissue conditioners (Quinn, 1985). In contrast, amphotericin B was completely ineffective when mixed with the tissue conditioner Visco-gel (Thomas and Nutt, 1978).

When the feasibility of a sustained-release delivery system for the treatment of denture stomatitis was studied, *in vitro*, by means of four antifungals (chlorhexidine gluconate, clotrimazole, fluconazole, and nystatin) incorporated into a tissue conditioner, it was found that all drugs were released from the tissue conditioner matrix with simultaneous inhibition of candidal growth (Schneid, 1992). Although these reports

indicate the *in vitro* inhibitory effects of antifungals on candidal growth, the nutrient-rich environment and the cleansing effect of the oral cavity are likely to minimize the beneficial action *in vivo* (Graham *et al.*, 1991; Okita *et al.*, 1991). Further, the dosages of the antifungals used in these *in vitro* studies appear to be high, costly, and impractical in clinical situations. In addition, the possible emergence of resistance organisms due to the constant presence of the drugs in the oral environment cannot be ruled out. Despite these caveats, slow-release agents and the related improvements in technology may be harnessed in future for the incorporation of antifungals into dental materials.

## (IV) *Prophylaxis of Oral Candidosis*

Those at greatest risk of fungal infection are compromised patients with HIV disease or receiving anti-neoplastic chemotherapy, radiotherapy, immunosuppressive therapy, or prolonged antibiotic therapy (Scully *et al.*, 1994; Lortholary and Dupont, 1997). Mucosal candidosis contributes markedly to the morbidity of HIV-infected patients, and, as stated earlier, it is the most common fungal infection in such populations (Wheat, 1993). Further, *Candida* is by far the most common fungal pathogen isolated from neutropenic patients and represents 90% of all fungal infections in this group (Varthalitis and Meunier, 1995). *Candida* is thus the most common target for antifungal prophylaxis in all types of immunodeficiencies. Hence, prophylactic anti-candidal regimens need to be frequently prescribed (Lortholary and Dupont, 1997). Such regimens could take the form of intermittent or continuous therapy. The mode of such therapy depends on several factors, including the severity of the underlying illness, frequency of recurrence of the fungal infection, concurrent antibiotic therapy (which promotes candidal growth), and the length of therapy, as well as the *Candida* species isolated from the patient.

The increasing resistance of C. *albicans* from HIV-infected patients undergoing long-term fluconazole therapy has become a major problem (Barchiesi *et al.*, 1994; Ruhnke *et al.*, 1994; Rex *et al.*, 1995a). Further, the recent discovery of C. *albicans* resistance to other antifungal drugs, such as amphotericin B and the other azoles, is worrisome (Kelly *et al.*, 1996; Le *et al.*, 1996; White *et al.*, 1998). Emergence of antifungal resistance in other non-*albicans* *Candida* species such as C. *krusei*, a phenomenon which is increasingly common, has also been reported (Pfaller, 1996; Samaranayake, 1996, 1997).

Often in the treatment of fungal infections, attention to the underlying cause will eliminate the need for prolonged or repeated courses of treatment (Scully *et al.*, 1994). The most important prophylaxis regimen against candidosis, therefore, is to minimize or eliminate factors which predispose to *Candida* infections (Lortholary and Dupont, 1997). However, intermittent or continuous antifungal treatment may be necessary when the underlying cause is incurable, as in HIV disease.

Prophylactic antifungal regimens should target not only the host mucosal surfaces but also the inanimate surfaces, such as prostheses, which may harbor yeasts and initiate re-infection. A classic example of this is denture plaque, which often contains *Candida* species. Therefore, to prevent *Candida*-associated denture stomatitis, in both healthy and compromised patients, denture cleansing that includes removal of *Candida* is a necessary prerequisite. Denture cleansers can be categorized according to their main components: alkaline peroxides, alkaline hypochlorites, acids, disinfectants, and enzymes (Budtz-Jørgensen, 1990). Of these, the proteolytic enzymes are found to be the most effective against *Candida*. Others have claimed that a denture-soaking solution containing benzoic acid completely eradicates C. *albicans* from the surfaces (Iacopino and Wathen, 1992), as do 0.2% chlorhexidine gluconate (Kamalakshi *et al.*, 1992) and a protease-containing denture soak (Odman, 1992). Though not very popular, these agents can be used as an adjunct to mechanical cleansing in preventing the recurrence of *Candida*-associated denture stomatitis.

## (V) *Susceptibility Testing*

*In vitro* susceptibility testing of antifungal agents is becoming increasingly important because of the introduction of new agents and the recovery of clinical isolates that exhibit intrinsic (primary) resistance or develop secondary resistance to the drug during therapy (White *et al.*, 1998). The primary objective of virtually all *in vitro* susceptibility tests is to facilitate the prediction of the efficacy of the antimicrobial agent on the outcome of infection caused by a specific pathogen. It should be borne in mind that, regardless of the purpose of such testing, results obtained in a simple, well-defined, and highly artificial *in vitro* system have intrinsic limitations in predicting the outcome of the clinical infections. Thus, only a modest correlation exists between *in vitro* results and clinical outcome, despite decades of experience with standardized testing methods (Pfaller *et al.*, 1997).

Susceptibility testing of fungi, including yeasts, presents numerous problems that are not encountered in the evaluation of bacterial susceptibility to antibiotics. Morphological variations, such as the yeast form and hyphal form, differences in growth rates, and optimal growth conditions have a profound effect upon the testing methods (McGinnis and Rinaldi, 1996). Susceptibility testing may be conducted in test tubes, petri dishes, or microtiter trays. Flow cytometry (Pore, 1994; Ramani *et*

**TABLE 4**

**Reported *in vitro* Minimum Inhibitory Concentration Values (μg/mL) of Common *Candida* Species against Widely Used Antimycotic Agents** (McGinnis and Rinaldi, 1996)

| *Candida* Species | AMP* | NYS | MCZ | CLT | KETO | ITR | FLU | 5-FC |
|---|---|---|---|---|---|---|---|---|
| C. *albicans* | 0.05-4 | 0.78-> 100 | 0.016-100 | 0.01-50 | 0.01-> 100 | 0.063-128 | 0.125-> 80 | 0.01-> 100 |
| C. *glabrata* | 0.05-> 100 | 1-> 50 | 0.016-64 | 0.25-25 | 1 | 2-128 | – | 0.01-> 100 |
| C. *guilliermondii* | 0.02-2 | 2-> 50 | 0.25 | 0.016-50 | 0.4-50 | – | – | < 0.02-10 |
| C. *krusei* | 0.05-> 6.25 | 0.5-25 | < 0.06-6.25 | < 0.5-1 | 0.1-10 | 0.125-0.5 | 32-64 | 0.1-> 25 |
| C. *parapsilosis* | 0.02-> 6.25 | 1-> 50 | 0.016-32 | 0.016-12.5 | < 0.12-> 64 | 0.06-> 128 | 0.5-4 | < 0.025-> 100 |
| C. *tropicalis* | 0.04-16 | 0.5-50 | 0.016-33 | 0.016-4 | < 0.12-> 64 | 0.13-> 128 | 0.5-> 64 | 0.01-> 100 |

* Abbreviations: AMP, amphotericin B; NYS, nystatin; MCZ, miconazole; CLT, clotrimazole; KETO, ketoconazole; ITR, itraconazole; FLU, fluconazole; and 5-FC, 5 fluorocytosine).

*al.*, 1997) and "E tests" (Dannaoui *et al.*, 1997) have been recently added to this repertoire. In addition, numerous other attributes—including the composition of the test medium, the form and concentration of the inoculum, the incubation temperature and duration, and the criteria used as end points—have a pronounced impact upon the MIC (Minimum inhibitory concentration) and MFC (Minimum fungicidal concentration) values obtained (McGinnis and Rinaldi, 1996). A simple technique to ensure intra-laboratory reproducibility is to incorporate a quality control (QC) isolate having known reproducible MIC and MFC values with each *in vitro* susceptibility test. Even with such strict control measures, the results often lack specificity and suffer from significant intra-laboratory variability.

Due to the difficulty in the interpretation of *in vitro* susceptibility data, the National Committee for Clinical Laboratory Standards in the USA (NCCLS, 1995) has recommended several standard antifungal susceptibility testing methods for yeasts. Correlation of the *in vivo* and *in vitro* activities of antifungal agents can be made, to some degree, by such standardization, and hence all workers in the field should refer to and adopt these techniques to yield universally comparable data (Anaissie *et al.*, 1994; Rex *et al.*, 1995b). Clinical application and interpretation of antifungal susceptibility tests have been reviewed recently (Pfaller *et al.*, 1997; Rex *et al.*, 1997) and will not be further discussed here. The MIC values of common *Candida* species against widely used antifungals are given in Table 4 (McGinnis and Rinaldi, 1996).

## (VI) *Post-antifungal* Effect (PAFE)

Suppression of yeast growth that persists following limited exposure to antifungal agents has been termed the "post-antifungal effect" (PAFE). This is an offshoot of a new concept, termed the "post-antibiotic effect" (PAE), that was introduced in relation to antibiotic use in the management of bacterial infections (Craig and Gudmundsson, 1996). It must be emphasized that the PAFE arises due to prior exposure of yeasts to antifungals for a short duration, rather than to continuous exposure for a longer period of time, as in the case of MIC determination.

Suppression of microbial growth is an inevitable accompaniment during the PAFE period. However, what is more interesting and intriguing is the associated weakening of microbial virulence which may directly or indirectly influence the host-parasite relationship (Shibl *et al.*, 1995). In clinical terms, the main significance of the PAFE is the impact that it may have on the characterization of the dosage regimens (Craig and Gudmundsson, 1996). For instance, antifungal agents with longer PAFE could be administered (against the infective organism) with longer dosing intervals and *vice versa*, without loss of efficacy and with lower frequency of adverse effects (Turnidge *et al.*, 1994).

Clinically, the goal with respect to treatment with antifungal agents is to maintain the drug concentration above the MIC for almost the entire dosing period (Scalarone *et al.*, 1991). Therefore, in the treatment of candidal infections, it would be desirable if the clinicians could rely on *in vitro* susceptibility tests to obtain critical information regarding antifungal dosage regimens. But, as discussed above, there is a lack of universally accepted standardized procedures which provide treatment guidelines that correlate well with the *in vivo* situation with regard to the most effective dosage regimen (Pfaller *et al.*, 1997). Furthermore, when determining MIC, one should remember that the fungi are continuously exposed to a constant level of the antifungal agent, whereas, *in vivo*, the organisms are exposed to fluctuating

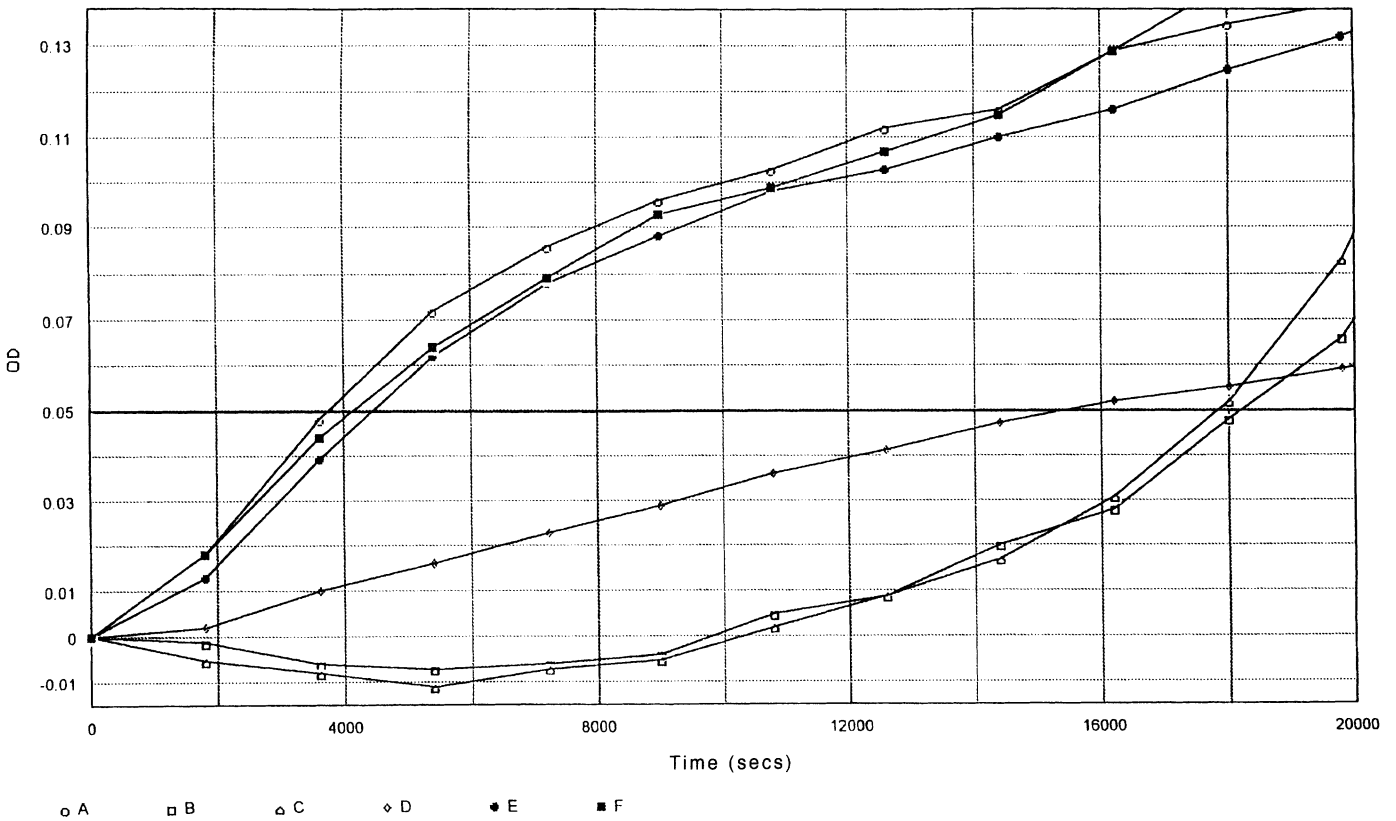


**Figure 2.** Growth curves of a *C. albicans* oral isolate following limited exposure to and subsequent removal of 5 antifungal drugs *in vitro*. Note the suppression of growth due to exposure to the polyenes and the DNA analogue. The x-axis denotes the time, and the y-axis denotes the optical density. The duration of PAFE was calculated according to the formula PAFE = T - C, where T was the time required for the relative optical density of the drug-exposed cell suspension to reach the 0.05 absorbance level after removal of the drug, and C was the time required for the relative optical density of the drug-free control cell suspension to reach the same absorbance level. Thus, T - C expressed the time in which the antifungal agent was capable of causing growth suppression of the organism following limited exposure to the drug (A, Control; B, Nystatin; C, Amphotericin B; D, 5-Fluorocytosine; E, Ketoconazole; and E, Fluconazole) (Ellepola and Samaranayake, 1998a).

levels of the drug (Scalarone *et al.*, 1992). This problem is further exacerbated in the oral environment due to the diluent effect of saliva and the cleansing effect of the oral musculature. Thus, the PAFE may be considered a relevant benchmark for determination of the dosage regimen of an antifungal which could be used in tandem with MIC values of the drug. The latter is the sole criterion currently used for this purpose.

The duration of the PAFE and even the presence or absence of the PAFE are influenced by several factors which essentially fall into three groups: the organism in question, the drug being tested, and environmental factors, which are summarized in Table 5 (Scalarone *et al.*, 1991; Turnidge *et al.*, 1994; Shibl *et al.*, 1995; Craig and Gudmundsson, 1996).

There is little information on the PAFE in *Candida* species. Perusal of previous research indicates that significant PAFE were produced by limited exposure of *Candida* species to amphotericin B and 5-fluorocytosine (Turnidge *et al.*, 1994). While PAFE of several hours' duration after exposure of C. *albicans* to 5-fluorocytosine has also been observed (Scalarone *et al.*, 1992), little if any PAFE has been observed with the imidazoles (Turnidge *et al.*, 1994) and the triazole fluconazole (Minguez *et al.*, 1994). Similar observations have been demonstrated on the PAFE of the polyenes, 5-fluorocytosine, and the azoles in 10 isolates of oral C. *albicans* (Ellepola and Samaranayake, 1998a; Fig. 2). More recently, PAFE of several hours following brief exposure to nystatin on non-*albicans Candida* species of oral origin has been reported (Ellepola and Samaranayake, 1999b).

The mechanisms by which antimicrobials produce the PAE in bacteria or the PAFE in fungi have not been clearly explained (Zhanel *et al.*, 1991; Shibl *et al.*, 1995). However, the three most commonly proposed mechanisms are: (a) the limited persistence of the drug at the microbial binding site, (b) the recovery from drug-induced non-lethal damage to cell structures, and (c) the time required for synthesis of new proteins and enzymes

**TABLE 5**

**Factors that Affect the Post-antifungal Effect (PAFE) of an Antimycotic Agent**

| Organismal Factors | Drug Factors | Environmental Factors |
|---|---|---|
| • Phenotypic and genotypic characteristics of the organism | • Mode of action of the drug | • Type of growth medium |
| • Inoculum size | • Exposure time to the drug | • pH of the growth medium |
| • Growth phase of the organism at the time of exposure | • Concentration of the drug | • Incubating temperature |
| | • Combined use of antimicrobials | • Mechanical shaking of the culture |

before resumption of cell growth (Craig and Gudmundsson, 1996).

It is instructive to examine, in some detail, the possible mechanisms by which antimycotic agents elicit a PAFE in *Candida* species. The polyenes alter the permeability of the yeast cytoplasmic membrane by binding to ergosterol, and a relatively prolonged period of time would be required for the cells to recover before active budding and multiplication could commence, thus eliciting a lengthy PAFE (Ellepola and Samaranayake, 1998a, 1999). On the other hand, the DNA analogue, 5-fluorocytosine, once within the yeast cell, is incorporated into RNA in place of uracil, with resulting abnormalities of protein synthesis and inhibition of DNA synthesis. The long PAFE usually observed with 5-fluorocytosine may represent the period in which there is abnormal protein synthesis, and suppression of DNA synthesis prior to commencement of normal cell division (Turnidge *et al.*, 1994; Ellepola and Samaranayake, 1998a). The azoles, on the other hand, cause alterations in fungal cell membranes by blocking the 14α-demethylation step in the biosynthesis of ergosterol. Interestingly, in contrast to the polyenes and the DNA analogue, the inability to elicit a significant PAFE by the azoles (Ellepola and Samaranayake, 1998a) may reflect the fact that the drug-induced effects are transient and readily reversible, or that the limited period of exposure is inadequate for these drugs to produce the desired effect.

It has been shown that, in bacteria, the PAE is not merely restricted to suppression of microbial growth. The other accompanying effects include changes in cell morphology (Hanberger *et al.*, 1993), inhibition of enzyme and toxin production (Shibl *et al.*, 1994), loss of adhesive properties and reduction of cell-surface hydrophobicity (Shibl *et al.*, 1994; Ramadan *et al.*, 1995), and increased susceptibility to host humoral and cellular immunity (Minguez *et al.*, 1994; Ramadan *et al.*, 1995). Many of these effects are probably overlapping and closely linked.

Despite these observations in bacteria, the impact of the PAFE on cellular attributes of *Candida* have not been extensively investigated thus far. However, a few studies documenting the effects of antimycotics on various pathogenic attributes of oral C. *albicans* isolates during the PAFE period are available. In one study, it has been demonstrated that the polyenes (nystatin and amphotericin B) and ketoconazole were capable of perturbing germ tube formation in C. *albicans* following brief drug exposure, while 5-fluorocytosine and fluconazole failed to exert such an effect during the PAFE period (Ellepola and Samaranayake, 1998b). In a separate study, it was found that all these drugs were significantly able to inhibit adhesion of *Candida* to denture acrylic surfaces during the PAFE period (Ellepola and Samaranayake, 1998e). With the exception of 5-fluorocytosine, similar suppression of candidal adhesion to buccal epithelial cells was elicited by the other antifungal agents during this period (Ellepola and Samaranayake, 1998d). Cell-surface hydrophobicity is a complementary factor involved in yeast adhesion to host surfaces and is considered an important pathogenic attribute of *Candida*. Our recent studies have disclosed that the polyenes and ketoconazole are capable of effectively minimizing the relative cell-surface hydrophobicity of oral C. *albicans* isolates during the PAFE period, while such an effect was not seen following limited exposure to 5-fluorocytosine and fluconazole (Ellepola and Samaranayake, 1998c). Further investigations, especially in relation to molecular aspects, are warranted to elucidate these phenomena of *Candida* during the PAFE period, and to translate these effects for therapeutic purposes.

## (VII) *Conclusion*

One major challenge in the management of patients with oral candidosis, especially those who are compromised, is to make a sensible selection from among the many antifungal agents now available on the market. The mode of action of the drug should be considered when an antimycotic is selected. This is an important factor in the treatment of patients chronically immunocompromised, such as those with AIDS, where azoles, especially fluconazole, should be the drug of choice. The polyenes should be routinely used in empirical therapy of primary oral candidoses, since the inappropriate use of the more useful azoles as the first drug of choice may result in eventual emer-

gence of resistant strains, thus rendering the drug worthless. Hepatotoxicity is common to most antifungals, and the potential for endocrine toxicity exists, particularly at high doses. Other considerations are the cost and cross-reactions with other therapeutic agents (*e.g.*, anticoagulants). However, by prescribing these agents according to their pharmacodynamic properties, one can achieve maximal antifungal activity while simultaneously minimizing patient exposure. Clearly, an ideal antimycotic for the treatment of oral candidoses is not yet available. But certain agents described above are better than others with respect to efficacy, tolerability, patient compliance, and cost-effectiveness. Most importantly, however, antifungal agents, however potent, may be rendered ineffective in the long term if the underlying predisposing factors are not attended to in the first instance.

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