A Pilot Study In The Use Of Topical Amphotericin B (Fungilin®) Lozenges for the Treatment of Oropharyngeal Candidiasis in HIV Positive Patients

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DEDICATION

This is dedicated to my parents Sandra and Lindsay Haynes

DECLARATION

This thesis is submitted in partial fulfilment of the requirements for the Degree of Master of Dental Surgery in the University of Adelaide.

This thesis contains no material which, except where due mention is made, has been accepted for the award of any other degree or diploma in any university. To the best of my knowledge, this thesis contains no material previously published or written by another person, except where due reference has been made in the text.

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MICHAEL A STUBBS

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ABBREVIATIONS

mg	milligram
*u*L	microlitre
ml	millilitre
HIV	Human immunodeficiency virus
KS	Kaposi's sarcoma
OHL	Oral hairy leukoplakia
HSV	Herpes simplex virus
URT	Upper respiratory tract infection
Psm	Pseudomembranous candidiasis
Eryth	Erythrematous candidiasis
Ang C	Angular cheilitis
C. albicans	Candida albicans
RPMI	Roswell Park Memorial Institute
CD4 positive	Cell expressing a CD4 receptor
CD8 positive	Cell expressing a CD8 receptor
AZT	Zidovudine
d4T	Stavudine
3TC	Lamivudine
I.M.V.S.	Institute of Medical and Veterinary Science

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CHAPTER 1

SUMMARY

SUMMARY

As the life expectancy of patients living with the human immunodeficiency virus (HIV) increases, clinicians can expect to see increasing numbers of patients with azole resistant candidiasis. The judicious use of prophylactic and therapeutic medications in this population, often requires the use of intravenous amphotericin B because of emerging azole resistant strains. The use of a topical antifungal agent, such as amphotericin B, in the treatment for oropharyngeal candidiasis in HIV patients has generally been avoided because at the standard dose their efficacy is limited. The purpose of this drug trial was to test the clinical efficacy of a non-standard dose of topical amphotericin B (Fungilin®), in treatment of the clinical signs and symptoms of oropharyngeal candidiasis in HIV positive patients.

Twenty patients who routinely attended for dental care at the Adelaide Dental Hospital were enrolled in the trial over an 18 month period. The entry requirements were that each patient be HIV positive, present with a form of oropharyngeal candidiasis according to the EC-Clearinghouse 1992 classification and give written consent to participate in the 12 week trial.

At time of enrolment each patient had an oral rinse taken to confirm the presence of *Candida*. Photography of all oral mucosa sites was undertaken and assessment of risk factors for the development of oral mucosal candidiasis was also evaluated.

Each patient was prescribed a sufficient topical amphotericin B (Fungilin®) 10 mg lozenges to suck intra-orally during waking hours. Patients were asked to evenly distribute the taking of the lozenges throughout the day and to suck them for a minimum of 10 minutes.

During the first two weeks all patients were taking eight lozenges daily. They were reviewed at weeks 1 and 2. They were then placed on a maintenance dose of four lozenges of topical amphotericin B daily for the remaining ten weeks.

At each review session intra-oral photography of all oral mucosal sites and an oral rinse was taken. Each trial participant was also assessed subjectively by questionnaire involving four questions asked at each review visit.

Twelve of the 20 patients completed the 12 weeks of the trial and remained clinically free of the signs and symptoms of oropharyngeal candidiasis. Of the remaining 8 patients, 5 also had clinical resolution of oropharyngeal candidiasis at the end of week 1 using the non-standard dose of eight amphotericin B lozenges daily.

In conclusion, this study demonstrated that irrespective of the immune status of a cohort of HIV positive patients, high dose topical amphotericin B was effective in resolving the clinical signs and symptoms of oropharyngeal candidiasis. This resolution occurred by seven days. A maintenance dose of four lozenges daily prevented relapse of oral candidiasis for the remaining ten week period of the trial in the twelve patients that completed the trial. Eight patients withdrew or were withdrawn from the trial. Clinical resolution of oropharyngeal candidiasis five of these patients occurred by the end of week one.

Current clinical management opinions do not favour the use of topical antifungals for the management of oropharyngeal candidiasis, except in the early stages of HIV infection. However, the results of the present study indicate that short term, high dose topical antifungals may be effective in the management of this condition irrespective of a patients HIV status.

CHAPTER 2
LITERATURE REVIEW

LITERATURE REVIEW

2. HIV/AIDS

2.1 The Human Immunodeficiency Virus

The Acquired Immune Deficiency Syndrome (AIDS) is caused by the human immunodeficiency virus (HIV). There are two HIV strains currently recognized. The first is HIV-1 which was isolated by the French Montagnier group in 1983. It was further characterized by both Gallo et al (1984) and the Montagnier group (1984). The HIV-1 virus has 9 subtypes A, B, C, D, E, F, G, H and O. Each of these HIV-1 subtypes is a unique strain. The strains show geographical differences in their distribution.

A second virus, HIV-2, was isolated from West African patients with AIDS or Aids Related Complex by the Montagnier group (1986).

The human immunodeficiency virus is an enveloped retrovirus with a unique enzyme, reverse transcriptase. This enzyme copies the viral ribonucleic acid (RNA) into deoxyribonucleic acid (DNA), which is inserted into the host cell chromosomes thus enabling the virus to replicate. HIV is a chronic infection and cannot be eradicated from host cells by any current anti-viral medications.

Cunningham et al (1997) reported on the structural similarities between the human immunodeficiency virus type one (HIV-1) and type two (HIV-2). The HIV virus has a lipid envelope which makes it susceptible to inactivation by drying, physical agents, and chemical agents. The viral surface has 72 glycoprotein receptors, each

second co-receptor CKR5 or CXCR4, also located in the cell membrane. The 'Fusion' peptide of glycoprotein 41 (gp41) is exposed, enabling the fusion of both viral and host cell membranes.

The HIV virus has different affinities for different cell types. The so called M-tropic strains represent HIV virus capable of infecting both the monocyte/macrophage cell lineage and the T-cell lineage, while other strains that replicate only in T cells are called T-tropic strains. The M-tropic strains are also known as 'non-syncytium-inducing' HIV virus. The T-tropic strains are also known as the 'syncytium-inducing' strains of HIV and usually occur late in the course of HIV infection. The co-receptor for the M-tropic strain is CKR5 also known as CC-CKR5 or CCR5. Work by Kelly et al (1996), using beta chemokines as antagonists to the CKR5 receptor, demonstrated the importance of these co-receptors in enabling the human immunodeficiency virus entering a host cell. The T-tropic strain co-receptor is CXCR4, formerly known as Fusin.

A review by Cunningham et al (1996) reported that CD4 positive T-cell lymphocytes are a primary site of viral reproduction, but that the human immunodeficiency virus infects three major cell types with functions central to the immune response. These are monocytes/macrophages, dendritic cells including the follicular dendritic cells found in lymph nodes and CD4 positive helper T-cell lymphocytes. The development of AIDS results from the immunodeficiency produced by HIV induced depletion or dysfunction of these cells. The human immunodeficiency virus also impairs the function of other cell types, including microglial cells in the central nervous system and epithelial cells of the gut. This can lead to conditions such as encephalopathy and diarrhoea respectively.

Cunningham et al (1996) further described the process of HIV viral replication as a chronic process involving billions of HIV virions being released daily (estimated to

take 2.6 days for a full cycle of replication within the host cell). These virions are combated by equally large numbers of CD4 positive T-cell lymphocytes. With time, gradual ineffectiveness of the host's immune system occurs because of immune cell death and reversal of the CD-4 positive T-cell lymphocyte to CD-8 positive T-cell lymphocyte ratio. In particular the host's cell mediated immune response is compromised.

2.2 Systemic Features Associated With HIV Infection

The clinical features of the seroconversion illness associated with infection by the human immunodeficiency virus are variable. They can include fever, arthralgia, lymphadenopathy and myalgia. Dermatological signs include an erythematous maculo-papular rash, mucocutaneous ulceration and alopecia. Neurological presentations can include headache and retro-orbital pain, meningoencephalitis or Guillian-Barre syndrome. Other signs of seroconversion may be oropharyngeal candidiasis, nausea and diarrhoea. A detailed list of the prodromal features associated with HIV infection, is presented in Appendix I.

The systemic manifestations of the human immunodeficiency virus infection are thought to be determined by the degree of immunosuppression existing at the time of patient presentation. In the early stages of infection, when the CD4 positive T-cell lymphocyte count is greater than 500 cells per uL of blood, various auto-immune and neurological signs and symptoms can present. These can include Reiter's syndrome, Polymyositis and Sjogren's syndrome. Neurological disorders such as Guillian-Barre syndrome, chronic demyelinating neuropathy or Bell's Palsy may also occur. This 'early stage' period can persist from 10 weeks to 5 years post-infection.

The 'intermediate stage' of infection is determined when the CD4 positive T-cell

- Viral: Cytomegalovirus, Herpes Zoster infection
- Fungal: Cryptococcosis, Candidiasis.

6.   The clinical presentation of certain tumors are considered AIDS defining in Australia and New Zealand, as reported by Marriott and McMurchie (1997):

- Kaposi's sarcoma.
- High and intermediate grade non-Hodgkin's lymphoma
- Cervical carcinoma in females.

2.3 Oral Manifestations Associated With HIV Infection

Signs of certain pathologies in the oral cavity can be a useful indicator in relation to the initial diagnosis of HIV infection and in relation to progression of the disease. In particular Rabeneck et al (1993) reported that the triad of oropharyngeal candidiasis, oral hairy leukoplakia and fever/night sweats are important markers in the context of the patient having full blown AIDS with a poor prognosis. A classification of oral lesions associated with HIV infection is presented in Table 1:

TABLE 1 Classification of Oral Lesions in HIV infection by the EC-Clearinghouse committee in 1992

GROUP 1	LESIONS STRONGLY ASSOCIATED WITH HIV INFECTION
	Candidiasis Erythematous candidiasis Pseudomembranous candidiasis Angular cheilitis
	Oral Hairy Leukoplakia
	Periodontal disease Linear gingival erythema Necrotising ulcerative gingivitis Necrotising ulcerative periodontitis
	Kaposi's Sarcoma
	Non- Hodgkin's Lymphoma

2.4 Candida

2.4.1 General description

Candida is a dimorphic yeast and is frequently encountered as a harmless commensal organism of the digestive system and the vaginal tract in females. *Candida* occurs in approximately 60% of healthy adults. *Candida* is referred to as an 'opportunistic' organism because it can rapidly become pathogenic if an underlying change occurs within a hosts normal microbial flora. This may occur when a patient is prescribed either corticosteroids or an antibiotic which alters the bacterial population present on mucosal surfaces. In such circumstances *Candida* can become dominant resulting in the clinical signs and symptoms associated with candidiasis.

Candidosis is the correct term to describe an infection by *Candida*. The suffix '-osis' is associated with mycotic infections, whereas the suffix '-iasis' is used to describe a parasitic infection, such as Amoebiasis. Unfortunately the term candidiasis has persisted in common usage and is often used synonymously with the more correct term candidosis. Rippon (1982) has suggested that the difference in terminology is geo-politically based, with candidiasis being a distinctly American term whereas candidosis is essentially European in origin. The terms Thrush and Moniliasis are also commonly encountered descriptions for *Candida* infections. The term candidiasis is used throughout this thesis to describe an infection by *Candida*.

Several terms are used when describing the morphology of a *Candida* organism. These are as follows:

1. *Pseudohyphae*: elongated yeast cells formed by polar budding constricted at the cell junctions. Pseudohyphae are usually joined to form chains and clusters.

2. *Germ-tubes*: the initial stage in a yeast to hyphal transition. Shepherd, Poulter and Sullivan (1985) describe a germ tube as hyphus like in appearance, but with no constriction at the mother cell junction.

3. *Blastospores*: represent the round or oval yeast stage. Blastospores are usually 3 - 4 um in diameter

Although *C. albicans* is the most frequent cause of superficial candidiasis in humans there are seven other members of the *Candida* genus which are of major medical importance. These are:

1. *C. albicans*
2. *C. tropicalis*
3. *C. glabrata*
4. *C. parapsilosis*
5. *C. stellatoidea*
6. *C. guilliermondii*
7. *C. krusei*
8. *C. pseudotropicalis*.

Of these, Scully and El-kabir (1994) reported that the most common

species in oropharyngeal candidiasis infection are *C. albicans, C. tropicalis* and *C. glabrata.*

In a patient with *C. albicans* infection the yeast's mainly derive from the patient's own endogenous reservoir in the mouth and intestinal tract. In some cases the infection is acquired from another person. Barehiesi et al (1995) showed that neonatal oral candidiasis is more common in infants born to mothers with vaginal candidiasis, suggesting that the infant internalizes some of the vaginal birth canal contents during birth or derives *Candida* organisms from the mothers hands.

Patients who have *Candida* as a commensal organism are called 'carriers' for that yeast. Carriage of *Candida* is a term used in the literature to describe the transfer of *Candida* from a carrier to another individual or transfer of *Candida* from one site to another in an individual carrier.

Melbye and Schonheyder (1985) have demonstrated a shift in the emergence of non-albican yeast species as the source for superficial oral candidiasis. This has been attributed to the use of antifungal agents such as Fluconazole, resulting in the selection of the fungal populations that have a greater resistance to the azole drugs in both HIV and other immunocompromised populations.

2.4.2   Oropharyngeal candidiasis in the immunocompetent patient.

2.4.2.1   Clinical classification of oropharyngeal candidiasis in an immunocompetent patient

There are many classifications of oropharyngeal candidiasis. These are based on either clinical or histological criteria. This variation is evident in the number of classifications for both 'normal' patients and those with HIV infection.

The clinical presentation of oropharyngeal candidiasis in an immunocompetent patient has been classified by Fotos and Hellstein (1992). This classification is as follows:

Acute pseudomembranous candidiasis (synonymously called 'thrush') is characterised by white-yellow plaques that can be easily wiped off the oral mucosal surface. The underlying mucosal surface is often erythematous or ulcerated.

Atrophic or erythematous candidiasis can present within the oral cavity as an acute or chronic form. The acute form sometimes corresponds to 'median rhomboid glossitis' and may be characterised by a burning sensation involving the tongue or mouth. Often a corresponding lesion is present on the hard palate. The chronic form of erythematous candidiasis is often seen clinically as erythematous and oedematous mucosa following the outline of a denture.

Chronic hyperplastic candidiasis presents within the oral cavity as a non-sloughable white plaque on the mucosal surface. Candidal hyphae are frequently found to be invading the epithelium rather than simply colonizing the surface.

Angular cheilitis or perleche has been reported by Russotto (1980) as frequently occurring in association with chronic atrophic candidiasis. Russotto further described angular cheilitis as being characterised by tenderness, erythema and fissuring of the corners of the mouth.

2.4.2.2 A histological classification of oropharyngeal candidiasis

Based on work from Odds (1988) and Nagai, Takeshita and Saku (1992) a histological classification of oropharyngeal candidiasis is as follows:

Superficial candidiasis is often characterised by white patches on the surface of the oral mucosa, tongue and elsewhere. The lesions develop into 'curd-like' patches that can be easily wiped off often revealing an erythematous or ulcerated mucosal surface. A swab will contain necrotic debris and desquamated parakeratotic epithelial cells, yeast cells and pseudohyphae. A biopsy will demonstrate the penetration of *C. albicans* yeast cells and pseudohyphae within the epithelium as far as the strateum spinosum. Oedema and micro-abscesses containing polymorphonuclear leukocytes are found in the outer layers of epithelium. The deeper parts of the epithelium

show acanthosis and the inflammatory response in the connective tissue comprises lymphocytes, plasma cells and neutrophils. The histology is not specific, apart from the identification of fungi present.

Locally invasive candidiasis is a term used to describe the penetration of fungal organisms into the underlying tissue. On mucosal/skin surfaces, necrosis of the overlying epithelium causes the mucosal surface to ulcerate. A pseudomembranous layer covering the base of the ulceration contains a matt of pseudohyphae embedded in fibrin and necrotic debris. The *Candida* organisms can infiltrate to deeper layers within the submucosa leading to haematogenous spread.

Systemic candidiasis is defined by Ellis (1994) as the presence of *Candida* organisms in the blood with or without visceral involvement. Hematogenous dissemination may then occur to more than one organ system with the formation of numerous microabscesses within the involved organ. Histopathologically, these multiple micro-abscesses within the affected organs exhibit *Candida* blastospores and pseudohyphae, a mixture of acute and chronic inflammatory cells and with an adjacent granulomatous reaction. This process is also referred to as 'Candida septicemia'. Carpentier, Kiehn and Armstrong (1981) reported the usual presentation in such cases is of a patient with a persistent fever that is unresponsive to broad-spectrum antibiotics. Up to 30% of patients with acute leukemia die of a systemic candidal infection.

2.4.3 Pathogenicity of *Candida*

Both Kauffman and Jones (1986), and Fotos et al (1992) have demonstrated that *C. albicans* can be isolated from the oral cavity in 60% of healthy immunocompetent patients without any pathological changes being clinically evident. There is controversy in the literature as regards differentiation between commensalism and disease. For this reason cytologic evidence of fungal pseudohyphae occurring simultaneously with clinical signs and symptoms remains the primary basis for the diagnosis of oral candidiasis. Lamey (1988) found that positive cytology in the absence of other diagnostic signs and symptoms does not imply mucosal candidiasis.

2.4.3.1 Non specific host factors associated with the prevention of oropharyngeal candidiasis in a immunocompetent patient

There are many factors that influence the presence and pathogenicity of oral *Candida* in the immunocompetent patient. Some of these can be attributed to saliva and its composition. For example, the salivary flow rate and its viscosity have a natural washing effects on *Candida* and bacteria from the oral mucous membrane surface. Challacombe (1994) reported that saliva also brings non-specific antimicrobial factors including lysozyme, lacteroperoxidases, histatins, calprotectin, and lactoferrin in contact with oral microbes. The effects of a lack of these non-specific salivary antimicrobial factors has been

reported by MacFarlane (1990) in relation to *Candida* infection commonly seen in xerostomic patients.

The composition of saliva also impacts on the presence and extent of *Candida* within the oral cavity of healthy, immunocompetent patients. Salivary constituents which are important in this regard include:

1.   *Iron* is essential for cell metabolism in both fungi and bacteria. Weinberg (1974) showed an increased susceptibility to both fungal and bacterial infection within the oral cavity when a host had a high serum iron level.

2.   *Lysozyme or muramidase* is normally found in high concentrations within the oral cavity. Tobgi, Samaranayake and MacFarlane (1988) showed lysozyme is present in saliva and gingival crevicular fluid and that it is derived from neutrophils. They also demonstrated that lysozyme is fungicidal to *Candida*, particularly to *C. krusei*. Hill and Porter (1974) found this enzyme can act, in association with Immunoglobulin A (Ig A), to stimulate phagocytosis and assist in agglutination of *Candida* organisms.

3.   The *histidine-rich polypeptides* found in saliva are called Histatins. Rayhan et al (1992) demonstrated that these histidine rich polypeptides, in combination with lysozyme, have an effective antifungal action. McCarthy

(1992) also reported that histatins are potent antifungal agents. However, although these proteins have the potential to protect against oral candidiasis, their exact mechanism of action has not been established.

4. *Lactoferrin* is an iron binding protein found in both parotid and submandibular saliva and in neutrophils. Tabak et al (1978) reported that the concentration of lactoferrin within saliva increases dramatically during inflammation of the oral mucosa and parotid salivary glands. Masson and Heremans (1971) showed lactoferrin has several modes of action against *Candida*. These include reducing the free iron available for metabolism by both bacteria and yeast's and enhancing the effectiveness of lysozyme by reducing the inhibitory effect of iron on its actions. Nikawa, Samaranayake and Tenouvo (1993) showed in some *Candida* species that lactoferrin created blebs within the yeast cell wall possibly interfering with yeast cell wall function.

5. *Lactoperoxidase* has been investigated by Klebanoff (1974) and shown to be antifungal in action by halogenating yeast proteins. Klebanoff also found lactoperoxidase caused aldehyde formation and oxidation of lipid sulfydryl groups.

6. *Salivary glycoproteins* were reported by MacFarlane (1990) to be similar to oral epithelial cell surface markers. *Candida* adhesion receptors recognize these

markers and bind to both the epithelial cell surfaces and the salivary glycoproteins.

As salivary glycoproteins are swallowed they assist in the removal of *Candida* organisms by blocking available yeast adhesion receptors that could have bound to host epithelial cells. MacFarlane (1990) also claimed that this process assisted phagocytosis of *Candida*.

7. *Calprotectin* was described by Brandtzaeg, Dale and Fagerhole (1987) as a calcium binding leukocyte protein. Calprotectin is normally produced in phagocytes such as monocytes and macrophages. The squamous epithelium from the oral mucosa also produces calprotectin. This salivary antimicrobial factor has been shown by Steinbakk et al (1990) to inhibit the *invitro* growth of various *Candida* species in addition to other microorganisms.

Other non-specific factors that have been reported to influence the oral *Candida* carriage rates and the incidence of oral candidiasis in healthy, immunocompetent patients are:

1. *Temporal variations*. In dentate patients Williamson (1972) demonstrated that the intra-oral density of *C. albicans* increased during sleep and was reduced by eating a meal or toothbrushing. Williamson also found overall oral *Candida* counts were at their highest in the morning.

In the edentulous patient, Budtz-Jorgansen (1990) found that the reverse applies. That is, patients who sleep without their dentures have the lowest count of oral *Candida* in the morning. This increases during the day because the acrylic bases denture harbor the fungal organisms

2. *Smoking*. There is considerable dispute in the literature about the impact of smoking on carriers of oral *Candida*. A study by Oliver and Shillitoe (1984) found that smoking has no effect on the *Candida* loads within the oral cavity. However, another study by Arendorf and Walker (1980) demonstrated a significant increase in the numbers of intra-oral *Candida* organisms within the oral cavity of smokers.

2.4.3.2 Cell mediated and humoral factors in the prevention of oropharyngeal candidiasis in immunocompetent persons

Additional host mechanisms that can influence the carriage and pathogenicity of *Candida* within the oral cavity are the cell mediated and humoral immune systems. Various cellular and humoral components of the immune system are important in this regard.

Phagocytosis is the process of recognition and attachment of a foreign particle, its engulfment with subsequent formation of a

phagocytic vacuole, and killing or degradation of the ingested material within the phagocytic cell. Intracellular digestion of the foreign material occurs via myeloperoxidase and lysosymal enzymes, reactive ions and oxygen radicals. Lehrer and Klein (1969) found the intra-cellular enzymes, myeloperoxidase and lysozymes play a major role in the digestion of phagocytosed *Candida*. These investigators emphasised the importance of these enzymes in patients with myeloperoxidase enzyme deficiency as such individuals often develop disseminated systemic candidiasis.

The phagocytic cells specializing in the intracellular digestion of *Candida* are neutrophils and macrophages. Shepherd et al (1985) concluded that the high incidence of disseminated candidiasis in neutropenic patients demonstrated the importance of neutrophils in protecting against *Candida* and other yeast organisms.

Lehrer and Klein (1969) found neutrophils to have the greatest capacity of all white blood cells to phagocytose *Candida*. However, there appears to be considerable variation in the ability of phagocytes to kill *Candida in vitro*. For example, Leijh et al (1977) showed variation ranging from 18.5% to 58%, in the ability of neutrophils to kill *Candida in vitro*. Davies (1972) demonstrated *in vitro* that not all *Candida* were killed by neutrophils and that some phagocytosed yeast cells grew successfully within neutrophils. The candicidal function of neutrophils *in vivo*, is less clear.

Following phagocytosis of *Candida* neutrophils produce 'tumor necrosis factor' (TNF). Ashman and Papadimitriou (1990) found the concentration of TNF is low and tends to be chemotactic rather than toxic. Further recruitment of neutrophils to the site of infection follows. Ashman and Papadimitriou further observed that in the presence of sub-optimal levels of interferon gamma the fungicidal action by neutrophils against *Candida* is markedly enhanced by TNF.

Mannoproteins from the cell wall of *Candida* organism have been demonstrated *in vitro* to have immunosuppressive activities against B and T-cell lymphocytes. Quinti et al (1991) concluded that a reduced cell mediated immune response to *Candida* mannoprotein may be one factor which explains the susceptibility of HIV infected patients to mucosal candidasis.

The cell mediated immune response dominates the humoral immune system in controlling *Candida* invasion of the oral mucosal epithelium. However, both the humoral and cell mediated arms of the immune system are activated against *C. albicans*. The humoral response, also called the secretory immune response, has many different modes of action. In the context of *Candida* and *Candida* infection this can involve:

1. Serum antibodies secreted against *Candida* enzymes such as phospholipases produced by the *Candida* hyphae. Pugh and Cawson (1977) showed that phospholipases are concentrated in the tips of fungal

hyphae and are localized to the immediate site of active tissue invasion.

2.   Epstein et al (1982) concluded that the secretory immune system also provides immunological protection to the oral mucosal surface. This is particularly so for immunoglobulin A (Ig A), which affords protection by inhibiting adherence and penetration of microorganisms and foreign proteins to mucosal surfaces. Vudhichamnong, Walker and Ryley (1982) demonstrated that secretory Ig A in saliva inhibits the adherence of *C. albicans* to epithelial cell surfaces.

Evidence to support these phenomena was later demonstrated by Fetter et al (1993). Fetter and colleagues found that parotid saliva in HIV positive patients contained less Ig A compared to a HIV seronegative control group, but greater Ig A levels than an AIDS group. Saliva from the HIV infected patients did however have greater IgG levels compared to the HIV seronegative control group. Fetter et al suggested that IgA levels may decline as a response to HIV infection itself, or may be secondary to a rise in IgG resulting from the polyclonal activation of B lymphocytes producing IgG.

Challacombe (1994) in a study directed towards analysis of the balance between commensalism and pathogenicity of *Candida* on the oral mucosal surface demonstrated

that strains of *C. albicans* and *C. glabrata* isolated from the oral cavity produced Ig A proteinases that were able to degrade Ig A1 and Ig A2. Challacombe postulated that those strains producing such proteinases may be more pathogenic on the oral mucosal surface than those that do not.

3.   Antibody directed cellular toxicity is important in the prevention of systemic candidaemia and appears to be intact in HIV seropositive patients. A study by Mathews, Burnie and Smith (1988) involving HIV positive patients showed that, irrespective of their disease stage, all subjects possessed antibodies to the 47 kDa antigen of *Candida*. The antibody against this particular antigen is thought to be important in preventing systemic spread.

4.   Both B and T-cell lymphocytes regulate the activity of neutrophils and macrophages by the release of lymphokines. Phagocytosis represents the primary mechanism of control of *C. albicans* once penetration within the oral epithelium has occurred. Ashman et al (1990) reported that the intrinsic phagocytic abilities of both neutrophils and macrophages against *Candida* are quite limited, and that the full expression of their candicidal effect is dependent on augmentation by lymphokines synthesized by T-lymphocytes. Ashman et al observed *in vitro*, that neutrophils or macrophages, in the presence of sub-optimal levels of gamma interferon

(INF-g), had greater candicidal activity when the phagocytes were also exposed to TNF.

2.5 Candida And HIV/AIDS

Felix and Wray (1993) described *C. albicans* as the most common fungal infection in HIV positive patients and that oral candidiasis is a clinical predictor of progression to AIDS. The general consensus within the literature is that oral candidiasis is a frequent and early oral manifestation associated with infection by the human immunodeficiency virus (HIV).

McCarthy (1992) reported the occurrence of oropharyngeal candidiasis in more than 90% of patients with AIDS. Studies by Coleman et al (1993) and Fetter et al (1993) have reported variation in the incidence of oropharyngeal candidiasis in HIV positive patients. These investigators have postulated that these differences may occur because of geographical factors and differences relating to the relevant proportions of hemophiliacs, intravenous drug users, homosexuals and heterosexuals within the HIV populations being studied.

2.5.1 *Candida* species found in HIV oropharyngeal candidiasis

In a study by Franker et al (1990), sixty percent of the HIV patients sampled had yeasts isolated from their oral cavity. *C. albicans* was the most frequent yeast cultured (84% of cases). The *C. albicans* biotypes 1, 3 and 13 were found to be most prevalent. Non-albicans *Candida* species formed the remaining 16%. Franker et al found the latter pathogens often included *C. tropicalis*, *C. glabrata*, *C. parapsilosis* and *C. krusei*. In a number of HIV positive patients these less frequently encountered *Candida* organisms were found in the same lesion as *C. albicans*.

Ancarni (1993) reported that the incidence of non-albicans *Candida* species associated with oral and respiratory tract candidiasis in 218 HIV patients was 22.1%. The most prevalent of these was *C. krusei* with a reported frequency of 10.6%, or half of the oral candidiasis caused by non-albicans species. Ancarni also noted that sixty five percent of the HIV positive patients with non-albicans *Candida* species cultured in this study had full blown AIDS.

2.5.2   Oral carriage of *C. albicans* in HIV positive patients

Both Torssander et al (1987) and Felix and Wray (1993) found that the carriage of oral *Candida* was greater in HIV seropositive patients than in seronegative patients. The prevalence of *C. albicans* increased in association with a reversing of the T-cell lymphocyte helper to suppressor ratio. However, there appeared to be no correlation between the extent of oral *C. albicans* carriage and the number of helper CD4 positive T-cell lymphocytes. Nevertheless, Melbye and Schonheyder (1985) reported that a correlation did appear to exist between an increased presence of *C. albicans* and increasing suppressor CD8 positive T-cell lymphocyte levels.

Other factors that influence the carriage of *Candida albicans* within the oral cavity of HIV positive patients are:

1.   Galli et al (1989) and Barone et al (1990) found greater carriage of *C. albicans* in intravenous drug users.

2. Franker et al (1993) observed greater colonization of *C. albicans* within the oral cavity of HIV positive patients diagnosed as group IV (Centre for Disease Control HIV disease staging protocol).

3. Inman et al (1990) and Carpenter et al (1990) observed that most oropharyngeal candidiasis occurred in HIV positive patients when the CD4 positive T-cell lymphocyte count was less than 300 cells per uL of blood. Oesophageal candidiasis occurred when the CD4 positive T-cell lymphocyte count was less than 100 cells per uL of blood. Fetter et al (1993) also found that the carriage of oral *Candida* increased within a HIV positive population when their CD 4 positive T-cell lymphocyte cell number was below 400 cells/uL and when there was a reversal of the CD4/CD8 T-cell lymphocyte ratio.

2.5.3 Phenotypic and genotypic variation of *C. albicans* in HIV oropharyngeal candidiasis

C. albicans has been shown to vary in its phenotypic expression with the progression of HIV disease. However, in a study by Quinti (1991), despite a change in *Candida* phenotype, the genotype of *C. albicans* persisted in a majority of HIV infected individuals examined. Studies by Hansclever and Mitchell (1961) and Nolan (1994) concluded that phenotypical switching of the same genetic strain of *C. albicans* could lead to a reduced susceptibility to azoles, amphotericin B, 5-fluorocytosine and nystatin. Nolan (1994) described other selective pressures for phenotypic switching of *C. albicans* including oral hygiene, age, socio-environmental factors, and gender.

Gallagher et al (1992) found many isolates (> 30%) of *C. albicans* from

Irish HIV positive and AIDS patients exhibited colony morphology variation on primary isolation and that these occasionally gave rise to additional colony variants on subculture. Preliminary studies indicated that some of these variant or switched derivatives exhibited decreased susceptibility to azole antifungal agents *in vitro*.

Gallagher et al concluded that in HIV positive and AIDS patients, where the levels of *C. albicans* can be very high, this variability could allow the generation of altered forms which are better adapted for survival in the oral cavity. Certain switched *C. albicans* derivatives can exhibit a variety of altered morphological, physiological, cell surface and genetic characteristics which may lead to variation in the yeast's ability to adhere to epithelial cells, to its antigenicity and to variation in such features as hyphal formation, cell size, growth rates, karyotype and genotype.

2.5.4 Serotyping *C. albicans* from HIV oropharyngeal candidiasis

In general comparison of studies serotyping of *C. albicans* is a problem because of the difficulty in standardization of results due to the availability of different biotyping methods. Williamson et al (1986) showed the benefits of using a particular biotyping test, the API 20C carbohydrate assimilation system, for yeast identification. This test divides the *C. albicans* species into two groups: types A and B.

The routine application of a reliable and standardized biotyping system for *Candida* isolates from HIV infected patients is highly desirable for the evaluation and localization of sources of infection, the determination of frequencies of changed biotype patterns, the selection of certain biotypes

during drug therapy, and the differentiation between therapeutic failure and re-infection in cases of recurrent or persistent candidiasis.

Recent studies by Brawner et al (1992) and Auger et al (1979) demonstrated that an increased pathogenic potential of *C. albicans* could be correlated with the *Candida* serotype B. For example, a greater incidence of 5-Fluorocytosine resistance was seen in serotype B *Candida*. However, Brawner et al (1991) considered that the commonly found serotype B species dominance in the oral cavity of HIV and AIDS patients was more likely due to an laboratory artefact.

McCullough et al (1984) found no correlation between the various biotypes and the clinical features of oral candidiasis. They also found no correlation between the biotypes of *C. albicans* and the clinical stage of HIV type 1 infection, or the number of CD 4 positive T-cell lymphocytes. Nevertheless, a study by Brawner and Cutler (1989) demonstrated that oral *C. albicans* isolated from immunocompetent individuals differ as a group from *C. albicans* isolates recovered from HIV patients based on the reactivity of isolates with an agglutinating immunoglobulin M (Ig M) class monoclonal antibody. This study provided the first convincing evidence for the presence of distinct types of *C. albicans* in immunocompetent and in HIV infected individuals.

2.6 HIV And Systemic Candidiasis

An increasingly compromised immune system in HIV positive individuals might suggest that superficial *Candida* infections could readily progress to systemic candidiasis. However, Reef and Mayer (1995) reported that systemic candidiasis is

an unusual feature associated with the HIV disease process. The exception is in neutropenic patients.

Oesophageal and dermal candidiasis represent a significant problem in HIV positive patients and systemic forms of therapy are required to treat these conditions. In oesophageal candidiasis the consequent dysphagia and retrosternal discomfort impacts upon the patient being able to maintain a proper diet. Treatment may require intravenous amphotericin B.

Evidence from animal models of systemic candidiasis indicates that humoral immunity is important in preventing systemic infection. Cutler (1976) found that congenitally athymic (nude) mice developed resistance to an intravenous inoculation of *C. albicans*. Further, Mourad and Friedman (1968) showed that hyper-immune serum provided protection against the development of systemic candidiasis when given to athymic (nude) mice. However, Pearsall, Adams and Bunni (1978) found the transfer of immune lymphocytes to athymic (nude) mice did not improve resistance to systemic *Candida* infection. Wray (1990) concluded that antibodies to *Candida* arising from polyclonal B cell activation, provided resistance to developing systemic candidiasis in HIV positive patients.

Arendorf and Walker (1980) concluded that the gastrointestinal tract is a common portal of entry for *C. albicans* into the body, and the colonization of *C. albicans* within the gut does pose a threat to the immunocompromised host. However patient's with HIV/AIDS, despite a diminishing CD4 positive T-cell lymphocyte population, do not appear to develop systemic candidiasis from the gastrointestinal tract mucosa.

2.7   HIV And Oral Candidiasis

The EC-Clearinghouse classified three categories of oral candidiasis associated with HIV infection. Previous classifications of oral candidiasis which apply to HIV negative individuals were not considered specific to the particular clinical presentations of oral candidiasis seen in HIV patients.

1.   *Erythematous candidiasis*

Presumptive criteria: A red area, usually located on the palate and dorsum of the tongue, but occasionally on the buccal mucosa. White spots and plaques may be seen, but these are not usually conspicuous.

Definitive criteria: At present there are no definitive criteria for this entity. However, the detection of *C. albicans* and/or the response to anti-fungal therapy may help to establish the diagnosis.

2.   *Pseudomembranous candidiasis*:

Presumptive criteria: White or yellow spots or plaques, which may be located in any part of the oral cavity. These plaques can be wiped off to reveal an ulcerated or erythematous mucosal surface, which may bleed.

Definitive criteria: The principle defining criterion is the response of lesions to anti-fungal therapy. Tests for the presence of *C. albicans* may include smears or culture.

3.   *Angular Cheilitis*: Can be associated with *C. albicans*, and may be seen in dentate patients with HIV infection.

Oropharyngeal and oesophageal candidiasis does occur with a high frequency in

HIV positive individuals. Carpenter et al (1990) observed that most oropharyngeal candidiasis occurred when the peripheral CD4 positive T-cell lymphocyte count was less than 300 cells/uL of blood and that oesophageal candidiasis occurred when the CD4 positive T-cell lymphocyte count was less than 100 cells/uL of blood.

The pseudomembranous form of oral candidiasis was found by Stenderup and Schondeyer (1984) to be the most common type of lesion seen in an investigation of a HIV population.

In a further study by Dodd et al (1991) these investigators concluded that both the erythematous and pseudomembranous forms of oral candidiasis are predictive of the progression to AIDS. Nielsen et al (1994) observed that HIV infected patients with oral candidiasis, either the pseudomembranous or erythematous types, were significantly more immune suppressed and will develop full blown AIDS at a faster rate compared to an HIV infected patient with normal oral mucosa. Nielsen et al also found that after a two year period there was no significant difference in the patient progressing to full blown AIDS irrespective of whether the erythematous or pseudomembranous types were present. Holmberg and Meyer (1986) in a retrospective study concluded that 10-20% of HIV patients in that study died as a direct consequence of fungal infections.

2.7.1 Diagnosis of oropharyngeal candidiasis in HIV positive patients

The diagnosis of oropharyngeal candidiasis is based on the presence of clinical signs and symptoms correlated with a cytology smear, biopsy or culture results. Torssander et al (1987) studying the carriage of oral *C. albicans* in HIV positive patients found high levels of these yeast's can be present within the oral cavity without any clinical signs being present.

Microscopically the presence of *Candida* can be determined by several methods. Mounting a smear of *Candida* in several drops of 10% potassium-hydroxide on a glass microscopic slide is a rapid method for assessing the presence of *Candida* pseudohyphae at chairside. The use of more specific fungal stains such as Periodic-Acid Schiff, Grocott's Methenamine Silver (GMS) and Calcofluor techniques can also be used to demonstrate fungal elements in smears or tissue sections. The diagnosis of oesophageal candidiasis is often based on a biopsy rather than a swab.

2.8 Management Of Oral Candidiasis In HIV/AIDS

2.8.1 Difficulties in therapeutic approaches

A study by Dupont and Drouhet (1988) found that oropharyngeal and oesophageal candidiasis rarely progress to become a life threatening disease. However, patients with HIV/AIDS need long term antifungal treatment so they can eat without discomfort and maintain adequate nutrition. Torssander et al (1987) and Boken et al (1993) have also observed that oropharyngeal candidiasis can act as a reservoir of infection for the development of oesophageal candidiasis.

The eradication of all *Candida* species from the mucous membranes is unlikely, or even necessary, as these organisms are often part of the normal oral flora. Current treatment regimens focus on the control of symptoms rather than attempts at eradication of the source.

Generally, because of the cost and high probability of developing a drug resistant strain, the long term use of any antifungal agent for prophylaxis

is contraindicated. Boken et al (1993) found that low dose therapy may also result in the development or retention of potentially resistant *Candida* strains. In addition, Sullivan et al (1993) showed that anti-fungal drug therapy often encourages the growth of less frequently found *Candida* species.

In the immunocompromised patient, it is important to recognize that different *Candida* species can be present in the same lesion. Thus addressing therapy towards one species of *Candida* may well not resolve the signs and symptoms. Strategies for prevention of endogenous candidiasis should also focus, in part, on methods of decreasing the mucosal colonization of *Candida*.

Complicating factors in the management of oral candidiasis are the interactions of other medications that patients might be taking and the effects of opportunistic infections acquired by the immunocompromised host. For example, Engelhard et al (1994) documented that Rifampin activates hepatic enzymes which reduces the concentration of serum ketoconazole. Lake-Bakaar et al (1988) found that achlorhydria also significantly reduces the absorption of ketoconazole and itraconazole.

In an immunocompetent patient a *Candida* infection can often be eradicated by use of a short term dose of topical antifungal agent. In an immunocompromised individual long term antifungal therapies are often required to enable resolution and prevent recurrence of *Candida* infection. HIV positive patients are often prescribed numerous antimicrobials, steroids and anti-inflammatory agents. As already stated, these can affect the prevalence, species and types of oral flora within these individuals.

Wingard et al (1991) reported that before the onset of the HIV/AIDS epidemic the incidence of fluconazole resistant *Candida* was rare. Wingard et al further observed that since the advent of HIV/AIDS an increase in the frequency of fluconazole resistant *Candida* organisms has been reported. The emergence of resistant strains is often correlated with advanced HIV disease. Ancarni (1993) suggested that because HIV patients were living longer and had deep immunodeficiency, the long term use of fluconazole particularly was selecting for populations of oral *Candida* resistant to azoles.

2.8.2 Mechanisms for the development of antifungal drug resistance

Dupont (1993) and Baily et al (1994) proposed several mechanisms for the development of anti-fungal drug resistance by oral *Candida*.

1. *The prevention of access to the drug's target*. This can develop by natural selection within the *Candida* population of the host. Odds (1993) reported evidence for this phenomenon with *C. lusitaniae* which is often resistant to amphotericin B. This organism rarely caused a disseminated mycosis until the use of amphotericin B in the 1980's increased. Boken et al (1993) have also reported fluconazole resistant *C. krusei* is becoming a problem particularly with patients receiving fluconazole prophylaxis.

2. *Modification of the drug target by mutation*. *In vitro* studies by Bart-Delabesse et al and Million et al (1994) reported antifungal drug resistance also occurred by a mutation within the existing *Candida* isolate causing a modification of the drug target. Molecular typing

methods have demonstrated that strains of *Candida*, can develop azole drug resistance. This evidence confirms that the long term use of antifungal azole therapy should be avoided.

3. *Increased production of the drug target*. Heald et al (1996) postulated that increased production of a drugs target can cause a reduction in the drugs effect. For example, the overproduction of the cytochrome P450 enzyme by *C. albicans* reduces the effectiveness of Fluconazole.

4. *Transmission of drug resistant species*. Infection by a resistant *Candida* species, either from distant sites within the same host or transmission from another host, can be associated with resistance to therapy. A study by Sangeorzan et al (1994) demonstrated that patients with vaginal candidiasis had *Candida* in other locations as well. These sites included the axilla, nails and infra-mammary folds. Sangeorzan et al also reported that clinical relapses in vaginal candidiasis can originate because of re-innoculation from these body sites. In this study a similar genetic strain of *Candida* species was found in both the vagina and other body sites.

Transmission of *C. albicans* between partners has been well documented by Greenspan et al (1992), Boerlin et al (1995) and Heald et al (1996). Iatrogenic transmission from instruments and the hands of health care workers has also been reported by Melbye and Schonheyder (1985).

The transmission of *Candida* strains between sexual HIV partners has also been demonstrated by Boerlin et al (1995). However, it is unclear whether

the azole resistant strains of *C. albicans* persist if transmitted to HIV negative individuals.

Nolan et al (1994) showed that recurrence of oropharyngeal candidiasis in HIV infected patient's can be due to the persistence of the same genotype through successive episodes of infection relapses, or because of re-infection with a new genotype.

2.8.3 Antifungal agents

There are many antifungal agents prescribed for the treatment and prevention of fungal infections. They are available in topical, oral and parenteral form. The therapeutic antifungal agents used in HIV negative patients have been widely used in the treatment of oral candidiasis for HIV/AIDS patients. The following list represents the most common antifungal agents used for the treatment of oral candidiasis in HIV/AIDS patients.

Amphotericin B

Amphotericin B is a polyene antibiotic also called a macrocyclic lactone (Ryley et al 1984). Macrocyclic lactones are produced by bacterial Streptomyces species. Two of these macrocyclic lactones, amphotericin B and nystatin, have been used for many years to treat oropharyngeal candidiasis.

Amphotericin B has useful clinical activity against *Candida* species, *Cryptococcus neoformans*, *Blastomyces dermatidis*, *Histoplasma capsulatum*, *Torulopsis glabrata*, *Coccidiodes immitis*, *Paracoccidioides braziliensis*, and *Aspergillus* species.

Warnock (1991) and Glauser (1993) both found that these drugs act on yeast ergosterol within the plasma membrane of *Candida*. Amphotericin B has a higher affinity for yeast ergosterol than for cholesterol in the mammalian cell membrane. The primary event that causes *Candida* cell death is believed to be the formation of an ergosterol-amphotericin B aggregate causing disruption within the cell membrane and increasing its permeability to cations and protons. A strong affinity between the hydrophilic portion of the polyene and ergosterol via hydrogen bonds allows the drug to penetrate the fungal plasma membrane. Goffeau and Slayman (1981) postulated that an increased permeability of the membrane from a combination of several polyene-sterol complexes formed a pore or channel that destroys the proton-motive force generated by the fungal plasma membrane ATPase. Goffeau and Slayman concluded that this process impedes the uptake of nutrients within the fungal cell.

Sokol-Anderson et al (1986) found that amphotericin B may induce the production of reactive oxygen metabolites causing oxidative damage to fungal cells. Medoff et al (1983) also observed that amphotericin B had some capability to enhance cell mediated immunity in the host.

Kerridge and Whelan (1984) described the amphotericin B molecule as rigid and rod-shaped with a hydrophobic and a hydrophilic end. The length of the molecule is approximately 2.2 nm which is similar to that of membrane phospholipids.

The side-effects of topical amphotericin B have been reported in Goodman and Gillman (1996) as occasional nausea or diarrhoea because of negligible absorption from the gastrointestinal tract. Schmidt-Westhausen

et al (1991) found topical amphotericin B treatment was useful in many superficial fungal infections confined to the strateum corneum or outer epithelial layers of the oral mucosa.

Intravenous amphotericin B has greater side-effects predominately fever and nephrotoxicity. Kennedy et al (1983) observed these systemic side-effects in 80% of patients who received intravenous amphotericin B for deep mycoses. The toxicity of intravenous amphotericin B is drug dose-dependent.

Because of its nephrotoxicity, intravenous amphotericin B has been produced in various forms such as phospholipid sheets, vesicles or liposomes with the aim of reducing this side-effect. Graybill (1996) concluded that the new lipid coated forms of amphotericin B allowed greater doses of intravenous amphotericin B to be given with less risk of nephrotoxicity.

Amphotericin B drug resistance is well documented particularly in association with yeasts cultured from the throat, stool and urine. Jensen et al (1993) observed that the use of prophylactic topical amphotericin B led to the development of resistance in *C. lusitaniae, C. parapsilosis* and *Trichosporon beigelii*. However, in general Odds (1996) noted that resistance to amphotericin B is rare, despite it being used for over 30 years. Odds further reported that strains known to be amphotericin B resistant possessed abnormally low levels of ergosterol in their cell membranes. This results in fewer sites for binding of amphotericin B to *Candida* thus reducing the therapeutic effectiveness of the drug.

Epstein (1990) reported that the use of topical antifungal agents such as

amphotericin B lozenges and nystatin suspension was only effective in the early stages of HIV disease. Cartledge (1993) concluded that topical amphotericin B had the disadvantage of multiple daily dosing with an unpleasant taste that may negatively affect compliance.

Fluconazole is a systemic antifungal agent, and like other azoles, interferes with sterol synthesis. It is classified as an ergosterol-biosynthesis inhibitor. Fluconazole is a fluorinated bis-triazole and is available in formulations for oral and intravenous administration.

Fluconazole is rapidly absorbed into the blood stream and its very low protein binding potential means that higher levels of fluconazole can circulate in most body fluids. Fluconazole is excreted unchanged in the urine.

Stevens, Greene and Lang (1991) found fluconazole was very effective in preventing recurrent oropharyngeal candidiasis in HIV/AIDS patients. The use of fluconazole has become an important niche in the treatment of mucocutaneous candidiasis in patients living with HIV/AIDS.

The mode of action of fluconazole has been described by Brammer, Farrow and Faulkner (1990). Fluconazole inhibits lanosterol C-14 alpha demethylase, the cytochrome P-450 dependent enzyme, essential for ergosterol synthesis of the fungal cell membrane.

Ketoconazole is a systemic antifungal agent and is also an azole drug. Ketoconazole is an doxalane imidazole derivative and is available in oral and topical formulations. It is optimally absorbed from the gastrointestinal tract when taken with food and is found in greater concentrations within

tissues compared to body fluids. Ketoconazole is inactivated in the liver and excreted in bile.

Graybill and Sharkey (1990) reported that ketoconazole has a similar action to fluconazole in that it inhibits the synthesis of ergosterol which is essential in the synthesis of yeast cell membranes. In addition Borgers and Vanden Bossche (1982) observed an effect of low concentrations of ketoconazole that may affect the pathogenicity of *Candida*. This effect was the prevention of yeast cells transforming into mycelial elements thereby preventing *Candida* escaping leukocyte phagocytosis.

Optimal absorption of ketoconazole from the gut occurs in an acid environment. Patients with HIV/AIDS frequently develop achlorhydria causing poor absorption of ketoconazole.

Itraconazole is a systemic antifungal agent and is a dioxolane triazole derivative. Itraconazole is currently available in oral capsule form only.

Itraconazole is incompletely absorbed from the gastrointestinal tract with optimal absorption when taken with food. Boken, Swindells and Rinaldi (1993) reported that itraconazole, like ketoconazole, is best absorbed in a acid environment. Many HIV and AIDS patients have achlorhydria resulting in low serum levels of itraconazole. Excretion of itraconazole and its metabolites is principally via the bile. The mode of action of itraconazole is similar to that of fluconazole.

Blatchford (1990) concluded that itraconazole is effective for the treatment of mucocutaneous candidiasis. However, van't Wout et al (1991) found the use of itraconazole in treating other forms of candidiasis was limited.

In the study by van't Wout et al they concluded that a major reason that itraconazole has not been used extensively as therapy for systemic candidiasis is because of the lack of an intravenous formulation, or a readily absorbed oral formulation.

5-Fluorocytosine is also a systemic anti-fungal drug. The 5-fluorocytosine is a fluorinated pyrimidine and interferes with nucleic acid synthesis. This antifungal drug is well absorbed from the gastrointestinal tract and is available in intravenous form.

Shepherd et al (1985) reviewed the literature associated with 5-fluorocytosine and reported that it was transported into susceptible fungal cells by cytosine permease and converted by cytosine deaminase to phosphorylated 5-fluorouracil. This metabolite is incorporated into fungal riboxynucleic acid. The result of this process is abnormal protein synthesis causing death of the yeast cell.

Miconazole is a systemic antifungal drug and is available in a range of topical, intravenous and parenteral forms. In the management of oropharyngeal candidiasis in HIV positive individuals, Scully et al (1994) described its use mainly as a topical agent. The antifungal action of miconazole is directed towards the cytochrome P450 enzyme of *Candida*.

Nystatin is a polyene antifungal antibiotic. Goodman and Gillman (1996) reported that although nystatin is structurally similar to amphotericin B and has the same mechanism of action it is more toxic and is not used systemically.

Nikkomycins are a systemic antifungal agent and are described by

Graybill (1996) as potent inhibitors of chitin synthase essential for cell wall synthesis. Graybill reported that nikkomycin Z had modest effects on *C. albicans* alone, but in combination with fluconazole, was a more potent antifungal agent than if either of these two drugs were used independently.

Pradimicin is a new class of systemic antifungal drug. Fung-Tome et al (1995) observed that analogues of pradimicin act by unknown mechanisms to inhibit multiple *Candida* species, *C. neoformans*, *Aspergillus* species, and other fungal pathogens.

2.8.4 Drug resistant oropharyngeal candidiasis in patients with HIV/AIDS

Fotos, et al (1992) concluded that as the life expectancy of patients living with HIV and AIDS increases clinicians can expect to see increasing numbers of patients with recurrent oropharyngeal candidiasis. In particular, fluconazole resistant candidiasis (Rex, Rinaldi and Pfaller 1995).

Wingard et al (1991, 1993, 1995), Rex et al (1995), and Price, LaRocco and Gentry (1994) also reported an increase in the incidence of oropharyngeal candidiasis due to non-*albicans* species of *Candida*. Many of these species, such as *C. glabrata* were resistant to fluconazole and appeared to be increasing in some hospitals.

2.9 Conclusion And Rationale For The Present Study

Oropharyngeal candidiasis is a debilitating fungal infection causing loss of taste, appetite, dysphagia and oral discomfort. Patients infected with the human immunodeficiency virus (HIV) are extremely prone to oral candidiasis in the order of 90-100 percent of cases. The presence of this oral infection results in a reduced quality of life.

Patients with intractable oropharyngeal candidiasis are currently treated with systemic antifungal medications. The development of resistant strains of *Candida* to these antifungal agents is becoming more common making the condition virtually untreatable except by intravenous amphotericin B. This drug has a high tendency to cause nephrotoxicity.

Decker and Masur (1994) concluded that because the common and long term use of tri-azoles may promote the development of resistance the administration of fluconazole for suppression of asymptomatic or non-life threatening *Candida* infections should be considered carefully.

Cartledge (1993), reflecting a general view within the literature, suggested the use of topical antifungal medications for the treatment of oropharyngeal candidiasis in HIV infected patients is largely avoided because of the frequency of daily use, their unpleasant taste, and because they have a limited efficacy at a standard dose. It is also widely accepted that topical antifungals are ineffective in the later stages of HIV disease and that they are associated with a poorer response and rapid relapse rate.

Review of the literature reveals that although there is widespread concensus that topical antifungals are of limited use in treating HIV associated oral candidiasis

there are no data describing the effect of short term, high dose topical antifungals in the treatment of this condition.

Such an approach could be a useful 'front-line' clinical protocol when oropharyngeal candidiasis is first diagnosed in HIV infection. Thus preserving the use of systemic azole antifungal agents for severe cases, including invasive or intractable oropharyngeal candidiasis.

The aims of the current study were:

1. To test the effectiveness of a non-standard dose of Amphotericin B (Fungilin®) 10mg lozenges as an effective treatment for oropharyngeal candidiasis in HIV positive patients.

2. To test its usefulness as a treatment modality in this immunocompromised group irrespective of the patient's HIV disease stage.

CHAPTER 3

MATERIALS AND METHODS

MATERIALS AND METHODS

3.1 Patient Sample

This study was conducted on a sample of South Australian HIV infected patients who routinely attended for dental care at the Adelaide Dental Hospital. The participants were all Caucasian and comprised nineteen homosexual males and one bisexual male. One bisexual and one homosexual patient were also intravenous drug users. Collectively, the patients enrolled in the trial presented a broad spectrum of clinical stages within the HIV disease process. Nine patients had full blown AIDS. There was also considerable diversity in both the medications that each participant was prescribed and the presence of opportunistic infections.

3.1.1 Inclusion criteria

There were four major requirements for entry into the trial:
(i) The patients could be male or female
(ii) All subjects had to be HIV positive
(iii) The subject must have clinically detectable oropharyngeal candidiasis.
(iv) Each subject must have provided written, informed consent to participate.

3.1.2 Exclusion criteria

There were three major exclusion criteria applicable to subjects enrolling in the trial or during the trial:
(i) Medical consent was not given by the patient's physician

(ii)   Relapses or failure to resolve oropharyngeal candidiasis during the trial after an initial period of 2 weeks.

(iii)   The development of a symptomatic reaction such as nausea or diarrhoea directly attributed to the use of amphotericin B lozenges.

3.1.3   Withdrawal criteria

A patient was able to withdraw from the trial:

(i)   If they chose to voluntarily withdraw at any time

(ii)   If any patient participating in the trial chose an alternative antifungal therapy such as the systemic azoles.

3.1.4   Trial approval

Approval to commence the use of high dose topical amphotericin B lozenges (Fungilin®) was received from both the University of Adelaide Ethics Committee on Human Experimentation–Project Number H/31/94 and the Royal Adelaide Hospital Ethics Committee–Project Number 950534

3.2   Amphotericin B Lozenges

The amphotericin B lozenges (Fungilin®) were generously donated by the Bristol-Myer-Squibb Company, Melbourne, Victoria.

The batch number of the trial lozenges was (B) 502241 with an expiry date of November 1996.

3.2.1 Fungilin® lozenge formulation details

The formulation details of Fungilin® lozenges supplied from Bristol Myer Squibb were as follows:

The active ingredient in Fungilin® lozenges is 10 mg of amphotericin B. Additional materials included:

(i)   Mannitol

(ii)   Acacia

(iii)   Stearic acid

(iv)   Sodium cyclamate

(v)   Saccharin sodium

(vi)   Polyvinyl alcohol

(vii)   Talc-purified

(viii) Orange flavourings

3.3 Patient Induction

Patients were given an information sheet (Appendix III) explaining the purposes of the drug trial and the requirements for entry into the trial.

A consent form (Appendix IV) was signed by each patient after they had read the information sheet and asked any questions they might have had about the trial. Each patient was advised to immediately contact the clinic during the trial should any concerns arise about using the amphotericin B lozenges or should any side-effects develop.

3.3.1 Trial entry

Each patient's oropharyngeal candidiasis was classified according to the 1992 European Committee Clearinghouse criteria as follows:

(i)   Pseudomembranous candidiasis

(ii)   Erythematous candidiasis

(iii) Angular cheilitis

In most cases, the presence of *Candida* was initially confirmed at chairside using a smear taken from the intra-oral sites. The smear was spread into a drop of ten percent potassium hydroxide, placed on a clean glass microscopic slide and examined microscopically (Figure 1).

Figure 1 *Candida* **pseudohyphae as viewed in a drop of potassium hydroxide at x400 magnification**
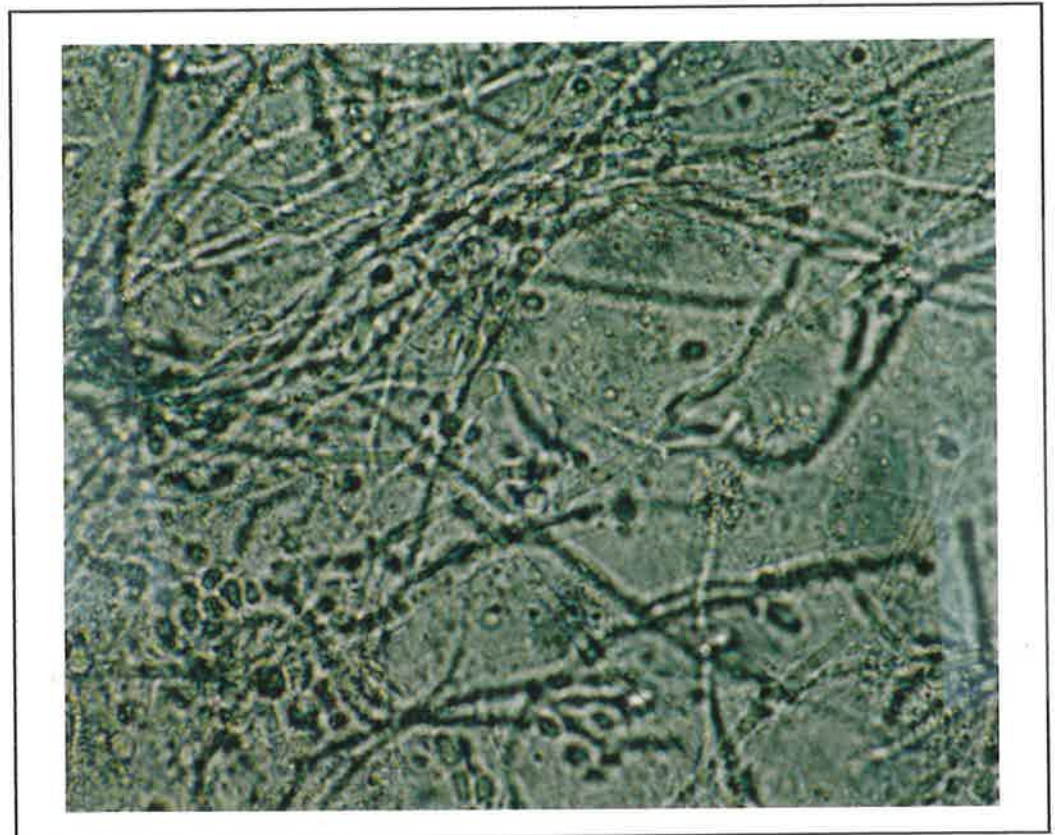
3.3.2 Oral rinse and fungal culture

In order to provide yeast samples for culture at the beginning and during the trial, subjects provided oral rinses.

The oral rinse medium was 20 ml of sterile, distilled water which had been autoclaved at 121 degrees Celsius for 10 minutes. Subjects rinsed their oral cavity for no longer than 30 seconds then expressed the fluid back into the sterile container.

At commencement of the trial a swab was also taken of clinically evident oropharyngeal candidiasis. Both the oral rinse and any oral swabs taken were sent to the Mycology laboratory at the Adelaide Women and Children's Hospital for culture, courtesy of Dr David Ellis.

The protocol for culture was as follows:
Both the oral swabs and rinses were plated onto Sabouraud's Dextrose agar containing Chloramphenicol and Gentamicin to eliminate oral bacteria within the culture. The agar plates where then incubated at 35 degrees Celsius for 48 hours. If *Candida* was present then colonies were evident within two days.

If colonies of *C. albicans* were present, they were semi-quantitatively scored as follows:

'+'   Indicated ten or less colonies of *C. albicans* that were present after incubation.

'++'   Indicated a moderate number 10-20 discrete colonies of *C. albicans* present on the agar plate.

'+++' Indicated greater than 30 colonies of *C. albicans* colonies present on the agar plate.

3.3.3 Photographic records

Intra-oral photographs of all oral mucosal surfaces were taken from each subject at the commencement of the trial and during each recall visit using a Pentax SFX camera with a ring flash (Pentax AF080 C). The lens was a 100 mm Macro lens and aperture settings of 11 and 16 were used. The film type used was Kodachrome 35 mm (ASA 64).

The sites of intra-oral photography were the left and right buccal mucosa, the hard and soft palates, the anterior floor of mouth, both lateral borders and the ventral surface of the tongue. Each mucosal surface was photographed twice using the alternate aperture settings.

3.3.4 Assessment of oral candidiasis risk factors

Subjects were asked a series of questions in order to assess and record the presence or absence of predisposing factors for oropharyngeal candidiasis. These questions were asked after the patient had enrolled in the drug trial. The questions related to the following:

(i)   Medications currently prescribed?

(ii) Smoking history. How many cigarettes smoked per day?

(iii) The subject's CD4 positive T-cell lymphocyte count, nearest to the time of trial commencement?

(iv) Whether the patient drank alcohol, and if so, how much was their average daily consumption?

(v)   Did the patient have a recreational drug habit such as marijuana?

3.4 Dispensing Of Amphotericin B Lozenges

The amphotericin B (Fungilin®) 10mg lozenges were stored at room temperature in the pharmacy of the Royal Adelaide Hospital and were dispensed to each patient enrolled in the trial by prescription only. For the purposes of confidentiality regarding each patient's HIV status each prescription was headed 'Topical Amphotericin B trial'. This enabled each patient to receive the non-standard dose and large numbers of lozenges in between each review visit without the need for subjects explaining why they needed the lozenges.

3.4.1 Dispensing of Amphotericin B lozenges at commencement of the trial

At trial commencement, each patient was prescribed fifty six (56) x 10mg amphotericin B lozenges from the Royal Adelaide Hospital pharmacy. Each patient was instructed to suck eight lozenges daily during waking hours. Each patient was also advised to evenly distribute the frequency of taking the amphotericin B lozenges throughout the day and to suck the lozenge for 15 minutes only.

3.5 Recall visits

3.5.1 Dispensing of Amphotericin B lozenges at each recall visit

During the second recall visit, each patient was prescribed a further fifty-six (56) x 10 mg amphotericin B lozenges. At the remaining three review sessions (weeks 4, 8 and 12), each patient was prescribed a maintenance dose of four (4) x 10 mg amphotericin B lozenges daily.

3. Any change in taste?

4. Any improvement in the symptoms since commencing the trial?

The same four questions were asked at each recall visit. The answers were compiled in a matrix format with an entry corresponding to the particular recall visit and question asked.

3.6 Sensitivity Testing Of *C. albicans* To Amphotericin B

Sensitivity testing of *C. albicans* to amphotericin B was undertaken on the first and last oral rinse taken from each patient. If *C. albicans* was cultured a colony was placed in RPMI media. This was then transferred to 20ml of distilled water creating a light suspension of *C. albicans*. One to two mls of suspension was then streaked over the surface of Sabouraud's agar and a disc impregnated with amphotericin B was placed in the streaked area. The culture was then incubated at 35 degrees Celsius for 48 hours and assessed under the light microscope for the presence or absence of *Candida* colonies.

CHAPTER 4

RESULTS

RESULTS

4.1 General Description

4.1.1 Sample

A total of twenty patients were enrolled in the study. Each patient was HIV positive and showed clinical evidence of oropharyngeal candidiasis. All subjects were males, with a mean age of 39 years 11 months. Nineteen of the patients were homosexuals, the remaining patient was bisexual. The bisexual patient and one homosexual patient were also intravenous drug users.

Each patient enrolled in the trial was at a different stage of their HIV infection. This was evidenced by the variability in the CD4 and CD8 T-cell lymphocyte counts and percentages between trial subjects and the variability in concurrent opportunistic infections present at time of entry into the drug trial.

4.1.2 Patient withdrawal

Eight patients withdrew from the trial, six because of non-compliance, and the remaining two because of xerostomia. Four of the eight patients withdrew in the first two weeks while the remaining four stayed in the trial for 3.5, 4, 8, and 9 weeks respectively.

4.1.3   Risk factors for oropharyngeal candidiasis

A common risk factor for the development of oral candidiasis in each of the patients was cigarettes (Table 2). Nineteen of the twenty participants smoked cigarettes. The number of cigarettes smoked per day varied from eight to a full packet. Smoking of marijuana was reported by four of the patients.

Although alcohol is not considered a risk factor for developing oropharyngeal candidiasis it was reported to be consumed on a regular basis in twelve of the twenty enrolled patients. Five patients consumed alcohol occasionally and three patients did not drink alcohol.

Four patients had an intra-oral prosthesis. Only one patient indicated that he soaked his dentures in Milton's solution (sodium hypochlorite) twice a week.

Five patients showed signs of oral xerostomia which was a side-effect of radiotherapy of the head and neck area in two patients. One of these two patients was treated for Non-Hodgkin's lymphoma of the hard palate and the other for oral Kaposi's sarcoma. In a further two patients xerostomia was attributed to the use of methadone. The remaining xerostomic patient showed evidence of HIV salivary gland disease.

Nearly all patients enrolled in the drug trial had poor oral hygiene and associated gingivitis with or without underlying periodontitis.

Table 2   Patient social profiles and possible risk factors for oropharyngeal candidiasis at the commencement of the trial

Patient	Social Profile	Oral risk Factors
1	Homosexual	C, A,
2	Homosexual	C, A, M
3	Homosexual	C, A, X
4	Homosexual	C, A, M, F
5	Homosexual	A, Y
6	Homosexual	C, A, F
7	Homosexual	C, M, A
8	Homosexual	C, A
9	Homosexual	C, A, M, X, P
10	Bisexual/IVDA	C, A, X,
11	Homosexual	C, A
12	Homosexual	C, A
13	Homosexual	A
14	Homosexual/IVDA	X
15	Homosexual	C, A
16	Homosexual	X
17	Homosexual	C, A, F and P
18	Homosexual	Nil
19	Homosexual	C, A
20	Homosexual	C, A

LEGEND			
A:	Alcohol consumption	IVDA:	Intravenous drug user
C:	Cigarettes	P:	Partial denture
X:	Xerostomia	OHL:	Oral hairy leukoplakia
Y:	Yoghurt (natural)	URT:	Upper respiratory infection
M:	Marijuana	HSV:	Herpes simplex virus
F:	Full denture	KS:	Kaposi's sarcoma

4.1.4 Opportunistic infections present at the time of trial commencement

Patients had a variety of oral opportunistic infections present at time of enrolment. The most common were oral hairy leukoplakia, Kaposi's sarcoma and herpes virus infections

Table 3 records the presence of any opportunistic infections at the time of

subject entry into the trial. The year of HIV diagnosis or seroconversion is also recorded (where known).

Table 3 **Opportunistic infections present in trial subjects at commencement of the trial. Year of HIV diagnosis/seroconversion (where known) is also recorded**

Patient	Other opportunistic infections at time of trial entry	Year of HIV diagnosis or seroconversion
1	Nil	Seroconversion: 1986
2	Nil	Seroconversion: 1992
3	Nil	Diagnosis: 1985
4	KS on feet	Unavailable
5	OHL bilateral	Unavailable
6	Herpes labialis	Unavailable
7	Nil	Diagnosis: 1991
8	KS intra-oral	Unavailable
9	HSV gingiva ulcer	Unavailable
10	Eczema, OHL	Seroconversion: 1983
11	OHL bilateral	Seroconversion: 1987
12	Nil	Seroconversion: 1990
13	OHL bilateral	Seroconversion: 1994
14	Nil	Seroconversion: 1981
15	HSV ulcer palate, OHL	Diagnosis: 1994
16	OHL bilateral	Diagnosed with AIDS 1993
17	Denture stomatitis	Diagnosed 1988
18	OHL bilateral, sinusitis, URT infection	Seroconversion: 1989
19	Nil	Diagnosis: 1994
20	Nil	Seroconversion: 1987

LEGEND	
A:	Alcohol consumption
C:	Cigarettes
X:	Xerostomia
Y:	Yoghurt (natural)
M:	Marijuana
F:	Full denture

The opportunistic infections that were present at the commencement of the trial for each subject are presented graphically in Figure 2.

Figure 2 Percentage of opportunistic infections present in all 20 subjects at commencement of the trial

Percentage of opportunistic infections present at start of trial

- Kaposi's Sarcoma
- Oral Hairy Leukoplakia
- Herpes Simplex Virus
- Sinusitis
- Denture Stomatitis
- Xerostomia

25% 10% 40% 15% 5% 5%
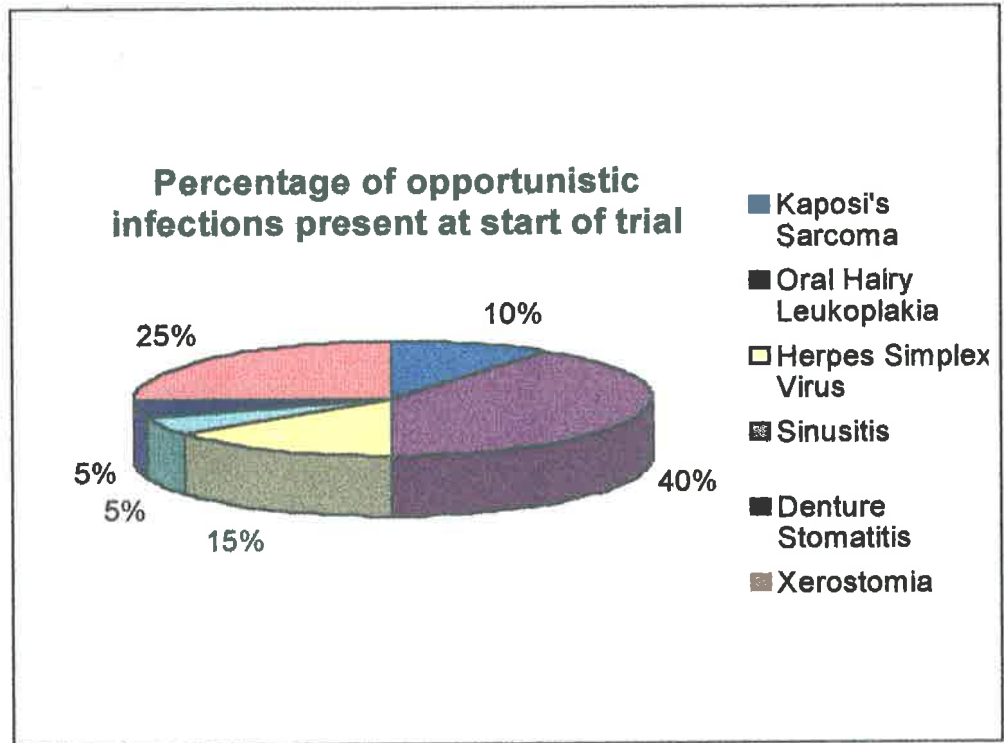
4.1.5 Questionnaire

During the trial each patient was reviewed five times over the period of twelve weeks. At each review session, the patient was asked the same four questions in order to subjectively evaluate each participant's progress throughout the trial. Questions 1 and 4 could be answered as either *Improvement* or *No Improvement*. Questions 2 and 3 could be answered as either *Yes* or *No*.

Table 4 **Summary of the answers provided from the twelve patients who completed the twelve week trial. Responses are recorded as a proportion of the 12 patients who completed the 12 weeks of the trial**

Review	Week 1	Week 2	Week 4	Week 8	Week 12
How does your mouth feel?	Improvement 11/12 No improvement 1/12	Improvement 11/12 No Improvement 1/12	Improvement 11/12 No Improvement 1/12	Improvement 11/12 No Improvement 1/12	Improvement 11/12 No Improvement 1/12
Any problems with the medication?	Yes 2/12 No 10/12	Yes 2/12 No 10/12	Ye 2/12 No 10/12	Yes 2/12 No 10/12	Yes 2/12 No 10/12
Any change in taste?	Yes 1/12 No 11/12	Yes 1/12 No 11/12	Yes 2/12 No 10/12	Yes 2/12 No 10/12	Yes 2/12 No 10/12
Any improvement since starting the trial?	Improvement 10/12 No Improvement 2/12	Improvement 10/12 No Improvement 2/12	Improvement 10/12 No Improvement 2/12	Improvement 10/12 No Improvement 2/12	Improvement 10/12 No Improvement 2/12

4.1.5.1 Questionnaire replies from the participants that completed the trial

Question one: *How does your mouth feel?* The only patient who reported no improvement throughout the trial reported no symptoms or discomfort at the commencement of the trial. This was despite the presence of pseudomembranous candidiasis on the patient's floor of mouth mucosa. The remaining eleven patients did report improvement, two indicating their tongue felt less 'furry'.

Question two: *Any problems with the medication?* Following the first week of the drug trial, three patients answered *'yes'* to this question. Patients 7 and 1 had diarrhoea and were concerned that this was caused by the amphotericin B lozenges. Patient 15 suffered nausea during the first week of the drug trial which he attributed to the amphotericin B lozenges. At the end of the second week patients 10 and 18 also answered *'yes'* to this question. Patient 10 had difficulty remembering to take eight lozenges daily and patient 18 had nausea and diarrhoea. The three patients in week one who had reported problems reported *'no'* to the same question in week 2.

In subsequent patient review there were no reported problems experienced with the medication.

Question three: *Any change in taste?* During the twelve weeks of the trial, patient 2 reported an improvement in taste because his tongue felt less 'furry'. At the fourth week, patient

18 reported a similar experience which persisted for the remainder of the trial. No subject reported an adverse change in taste associated with the use of amphotericin B lozenges.

Question four: *Any improvement in the symptoms since commencing the trial?* Both patients 7 and 12 reported no improvement throughout the trial as both had no symptoms at trial commencement. The remaining ten patients reported improvement from week one.

4.1.5.2 Questionnaire replies from the participants who were withdrawn from the trial

A total of eight patients withdrew from the trial. Patients 13, 16 and 19 withdrew after the first week. Patients 8, 3, 9, 4 and 11 withdrew after two weeks, three and half weeks, four, eight and nine weeks respectively.

Question one: *How does your mouth feel?* Patient 8 reported no improvement during the first week of the trial, but did report improvement after the second week. The remaining seven patients reported improvement in the first week.

By week two patient 3 reported no improvement because he was xerostomic and unable to effectively suck the lozenges. The remaining four patients indicated that their mouths felt better.

At week four, patient 9 reported no improvement. This patient

was non-compliant and despite his insistence on staying in the trial he presented with extensive pseudomembranous candidiasis on each recall visit. Accordingly, using the criteria for patient withdrawal from the trial, patient 9 was prescribed fluconazole and withdrawn.

At week eight patient 4 reported improvement despite pseudomembranous candidiasis being present under the fitting surface of his full upper denture. Patient 4 refused to remove his denture when sucking the amphotericin B lozenges intra-orally despite being advised to do this. After week eight he failed to attend further review sessions.

Patient 11 had ceased taking the amphotericin B lozenges at week seven and had a major relapse on review at week eight (Figs 3, 4 and 5). Despite his enthusiasm to remain in the trial and a further two weeks of using four lozenges daily. Reduction but not elimination of his oral pseudomembranous candidiasis was evident. Patient 11 was withdrawn from the trial at week nine and prescribed fluconazole.

Figure 3: Patient 11 at week 4. Clinically there was no evidence of any oral candidiasis. OHL (arrows) is present on the lateral tongue surface
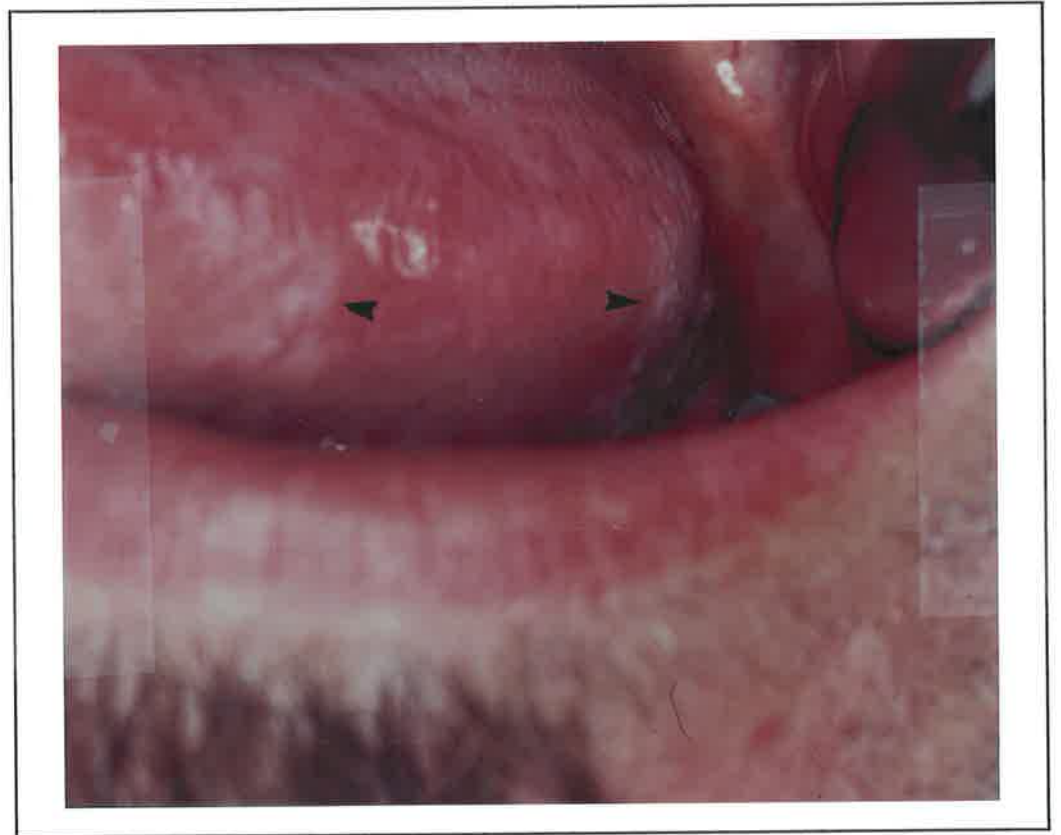
Figure 4 Patient 11 at week 8. There was pseudomembranous candidiasis (arrows) present throughout his oral cavity
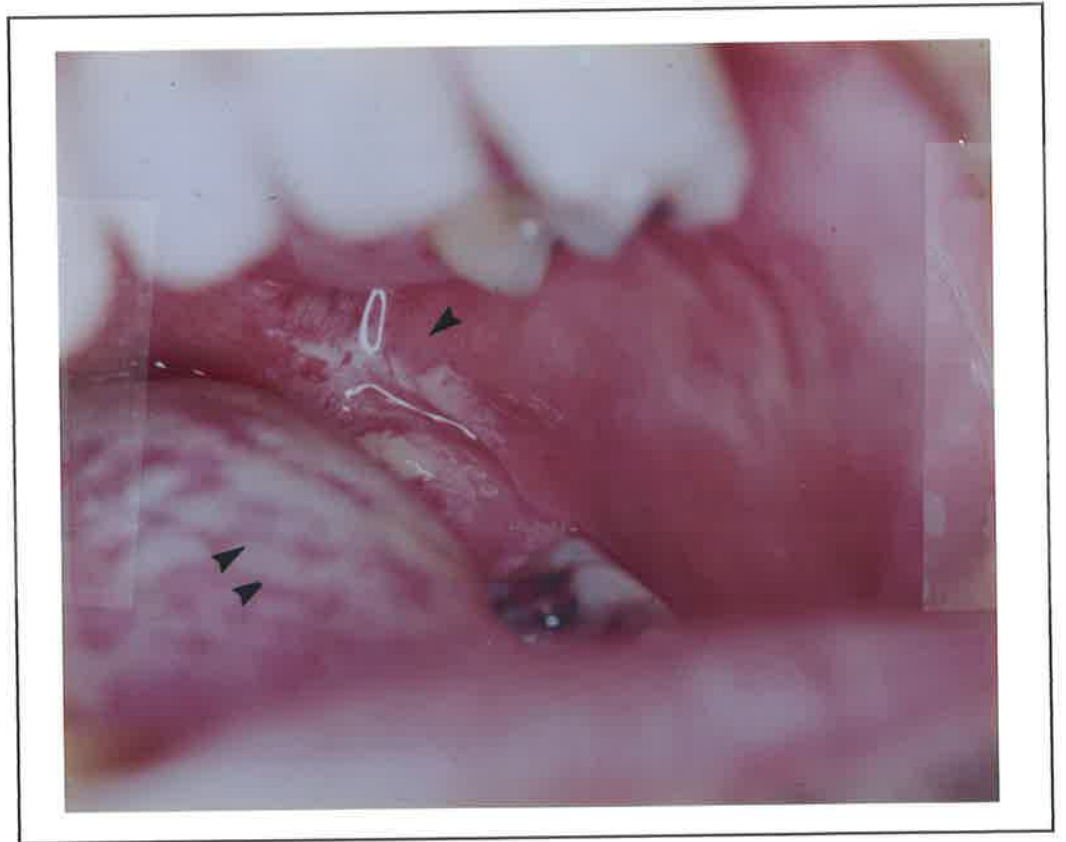
Figure 5 Patient 11 at week 9. Pseudomembranous candidiasis (arrows) was still present, despite the subject resuming the Fungilin® lozenges at the standard dose of four lozenges per day
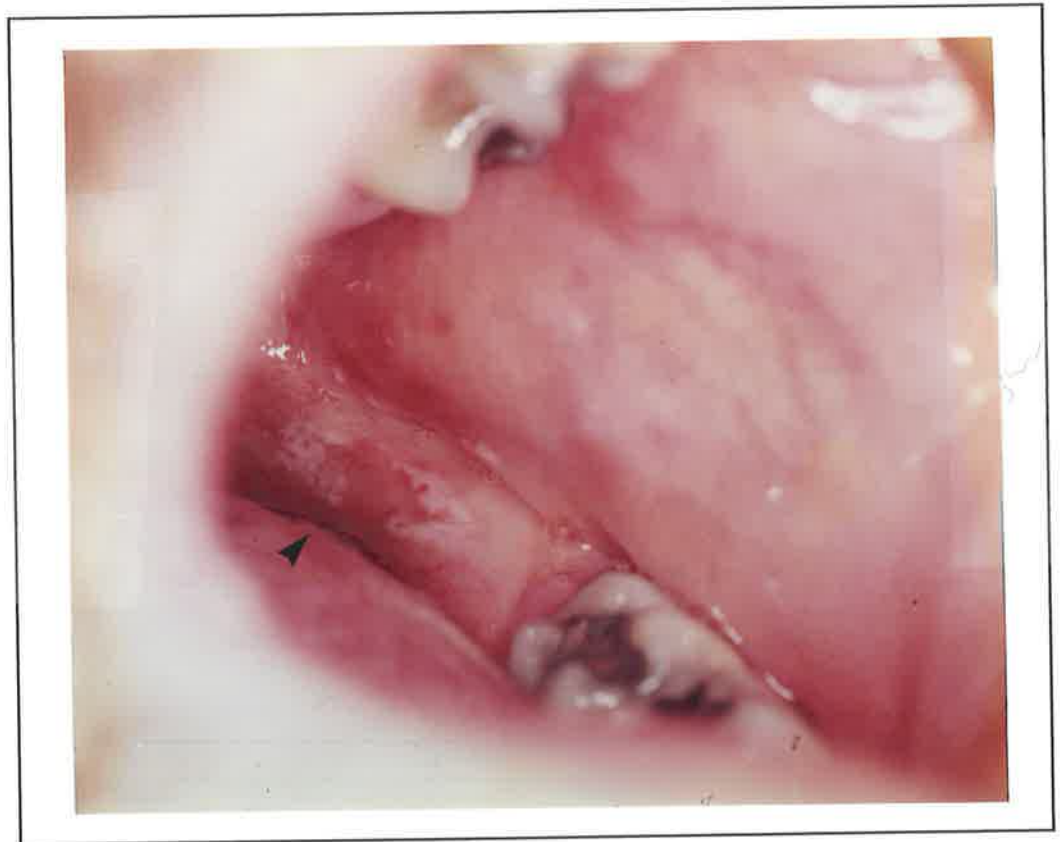
Question two: *Any problems with the medication?* Patients 3 and 16 were xerostomic and unable to dissolve the amphotericin B lozenges intra-orally. Patient 16 withdrew after the first week, but patient 3 declined withdrawal until week three and a half.

At week two patient 8 reported that the lozenges tasted terrible and he felt 'bloated'. He withdrew from the trial. At week four, patients 4 and 9 found it difficult to remember to take the lozenges. By the eighth week patient 4 again commented on having a problem remembering to take the lozenges.

Question three: *Any change in taste?* During the first week no improvement or change in taste was reported by any of the patients. At week four patient 4 reported a diminished taste to milk only.

Question four: *Any improvement since starting the trial?* At week one patient 11 reported no improvement despite having no clinical evidence of oropharyngeal candidiasis at week one. Patient 16 reported no improvement because of his xerostomia, and patient 8 answered this question with 'no improvement' because he was unaware of any symptoms from oral candidiasis at the commencement of trial.

At week two patient 3 who was also xerostomic, reported 'no improvement'. At week four patient 9 reported 'no improvement' and by week eight, patients 11 and 4 also reported 'no improvement' since starting the trial. As already

stated, patient 11 had ceased taking the amphotericin B lozenges at week seven and by review at week eight had pseudomembranous candidiasis extending to 70% of the oral mucosal surface.

Table 5   **Summary of the response to the questionnaire from the eight patients who withdrew from the trial. The responses are recorded as a proportion of the eight patients. At week 12 all eight patients had withdrawn from the study**

Review	Week 1	Week 2	Week 4	Week 8	Week 12
How does your mouth feel?	Improvement 6/8 No Improvement 2/8	Improvement 4/8 No Improvement 1/8	Improvement 2/8 No Improvement 1/8	Improvement 1/8 No Improvement 1/8	All patients had withdrawn
Any problems with the medication?	Yes 2/8 No 6/8	Yes 1/8 No 3/8	Yes 2/8 No 1/8	Yes 2/8 No 0/8	All patients had withdrawn
Any change in taste?	Yes 6/8 No 2/8	Yes 1/8 No 4/8	Yes 1/8 No 1/8	Yes 0/8 No 2/8	All patients had withdrawn
Any improvement since starting the trial?	Improvement 5/8 No Improvement 3/8	Improvement 4/8 No Improvement 1/8	Improvement 2/8 No Improvement 1/8	Improvement 0/8 No Improvement 1/8	All patients had withdrawn from trial

4.2 Candida

4.2.1 Clinical features at commencement of the trial

The presence of oropharyngeal candidiasis was an entry requirement for enrolment into the drug trial Of the twenty patients enrolled (see also Figure 6):

1. Nine patients presented with pseudomembranous candidiasis only (Figure 7)
2. Five patients presented with both pseudomembranous and erythematous candidiasis
3. One patient presented with pseudomembranous and angular cheilitis
4. Two patients presented with pseudomembranous and oesophageal symptoms
5. Two presented with erythematous candidiasis only (Figure 8)
6. One presented with erythematous candidiasis and angular cheilitis (Figure 9)

Figure 6   The types of oropharyngeal candidiasis present for each patient at time of enrolment into the trial

The incidence of oropharyngeal candidiasis at commencement of the trial
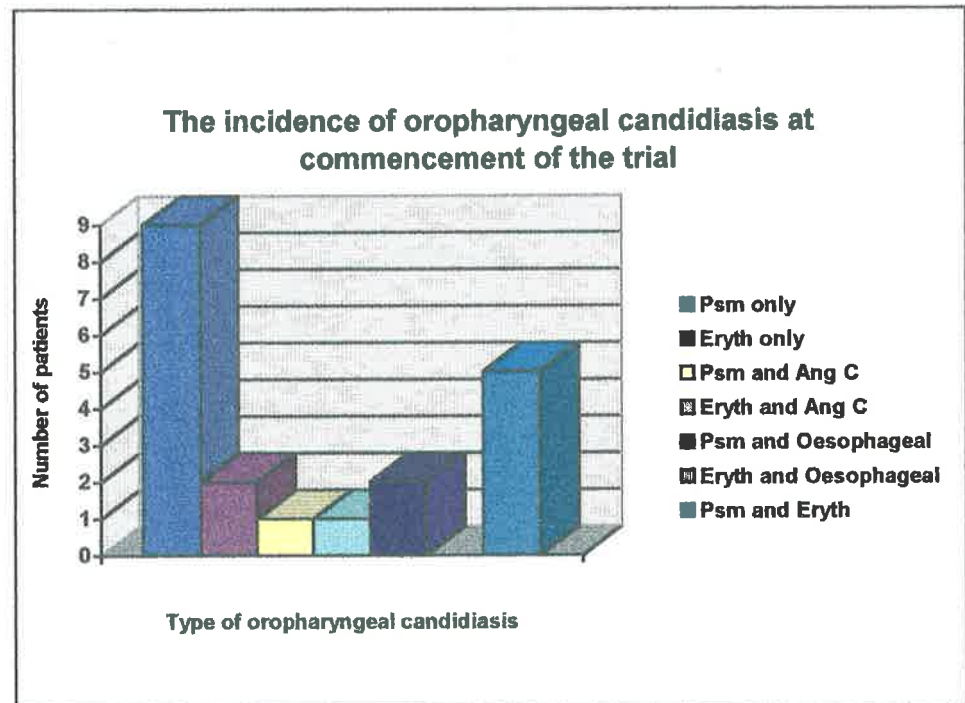
Legend	
Psm	Pseudomembranous candidiasis only
Eryth	Erythematous candidiasis only
Psm and Ang C	Pseudomembranous candidiasis and angular cheilitis
Eryth and Ang C	Erythematous candidiasis and angular cheilitis
Psm and Oesophageal	Pseudomembranous candidiasis and oesophageal symptoms
Eryth and Oesophageal	Erythematous candidiasis and oesophageal symptoms
Psm and Eryth	Pseudomembranous and erythematous candidiasis

Figure 7 An example of pseudomembranous oropharyngeal candidiasis (arrows) at commencement of the trial in patient 9
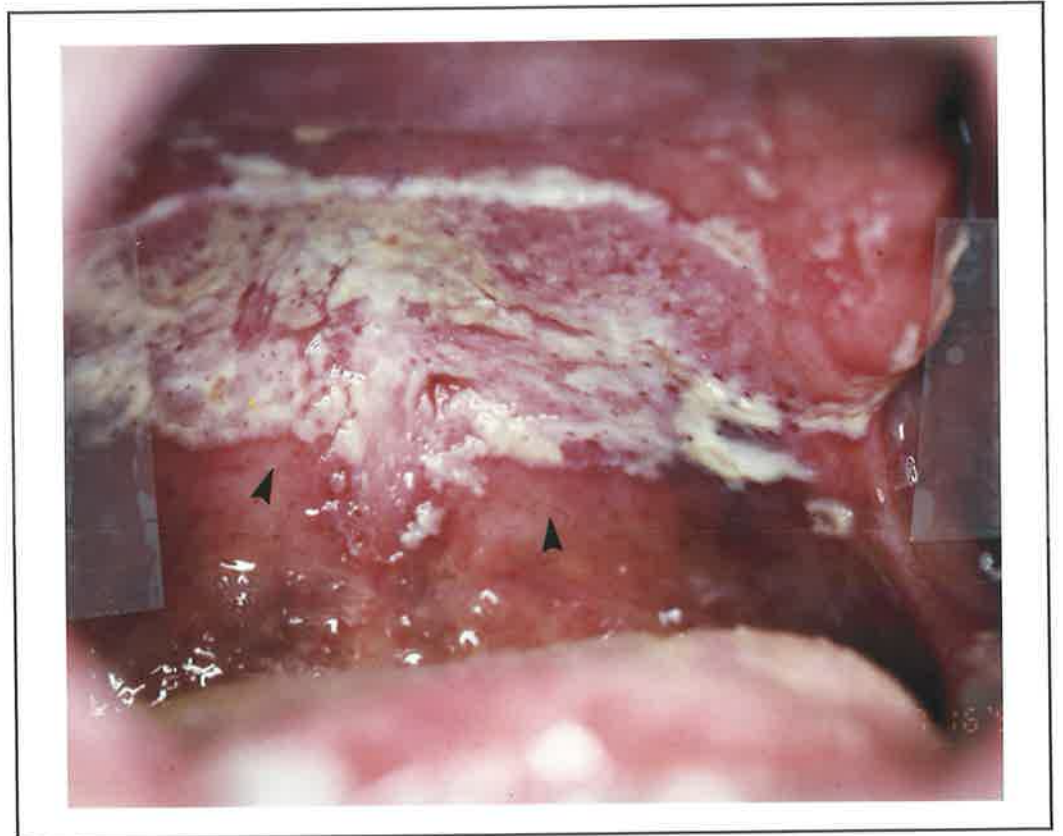
Figure 8 An example of erythematous oropharyngeal candidiasis (arrows) at the commencement of the trial in patient 2
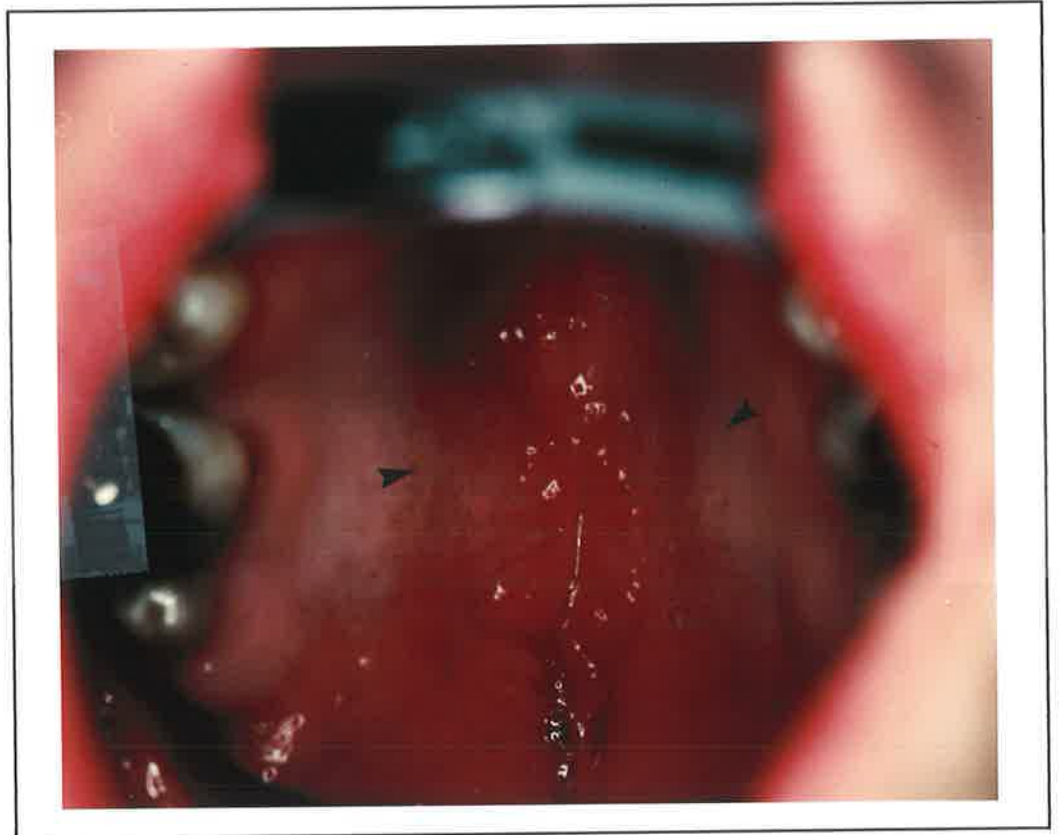
Figure 9 An example of angular cheilitis (arrows) of the lower lip
observed at the commencement of the trial in patient 6

4.2.2 Clinical features at completion of the trial

No signs of oropharyngeal candidiasis were evident in the twelve patients who completed the twelve week topical amphotericin B lozenge trial. Figures 10 and 12 represent the oropharyngeal candidiasis present at commencement of the trial from patients 5 and 1 respectively. Figures 11 and 13 represents the same oral mucosal sites at completion of the trial, from patients 5 and 1 respectively.

Figure 10 Pseudomembranous oral candidiasis (arrows) on the floor of mouth in patient 5 at the commencement of the trial
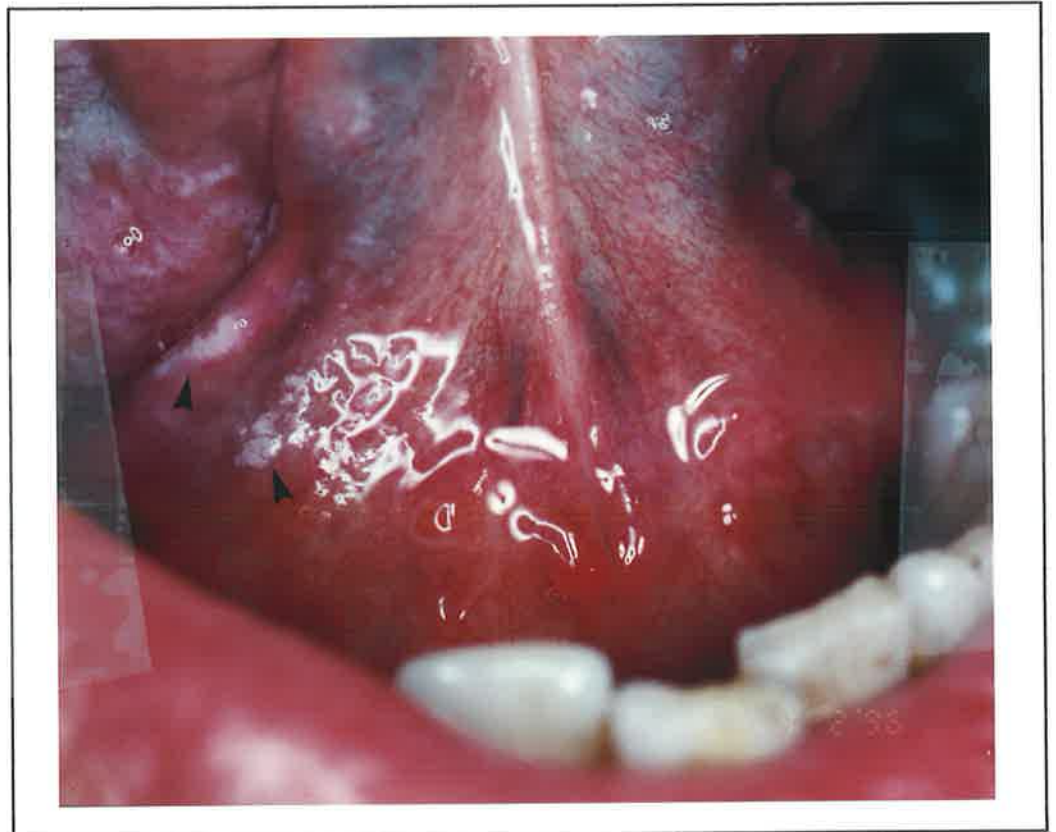
Figure 11 Resolution of the pseudomembranous oral candidiasis on the floor of mouth in Patient 5 at completion of the trial. No relapse occurred throughout the 12 weeks of the trial
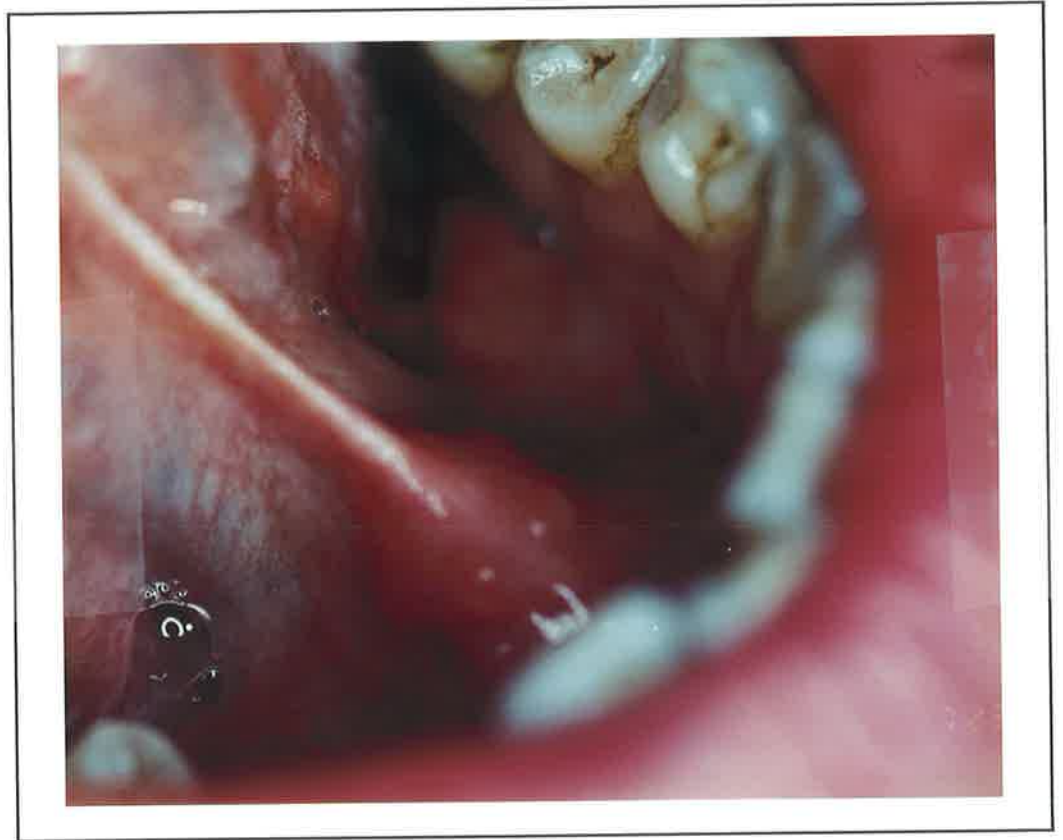
Figure 12 Patient 1 with erythematous oral candidiasis (arrows) of the hard palate at the start of the trial

Figure 13 **Patient 1 at completion of the trial. Resolution of erythematous oral candidiasis was maintained for the 12 week trial duration**
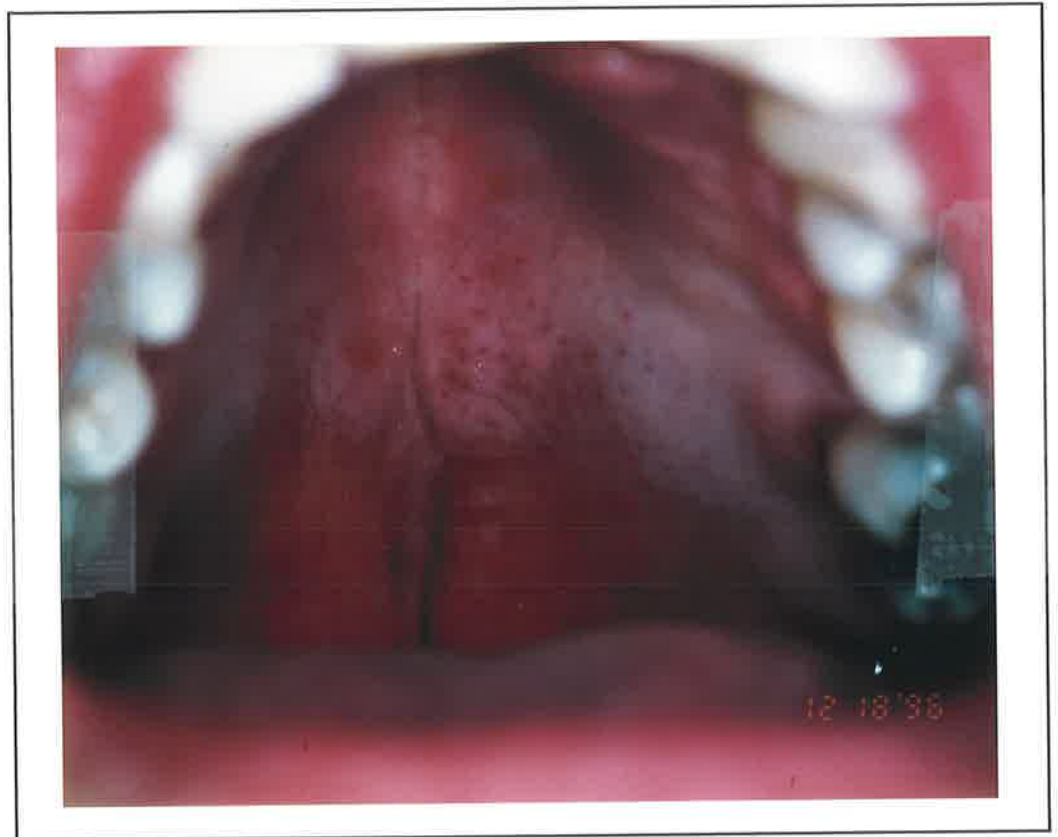
In five of the eight patients who withdrew from the trial, the non-standard dose of topical amphotericin B had resolved all of the clinical signs of oropharyngeal candidiasis by the end of week 1. Figures 14 and 15 shows patient 19 at the commencement and end of the trial. Figures 16 and 17 represent the same oral sites from patient 13 at the start of the trial and after one week of the trial. The remaining three patients who did not show clinical resolution of oropharyngeal candidiasis were xerostomic and unable to suck the Fungilin® lozenges effectively.

Seventeen of the twenty patients had effective resolution of their clinical signs and symptoms of oropharyngeal candidiasis by the end of week one.

Figure 14 Pseudomembranous candidiasis (arrows) on the dorsal surface of the tongue from patient 19 at the commencement of the trial

Figure 15 Patient 19 at week 1. Resolution of the
pseudomembranous oral candidiasis is evident

Figure 16 Patient 13 erythematous oral candidiasis (arrows) of the hard palate at the commencement of the trial

Figure 17 Patient 13 at the end of week 1. Resolution of erythematous candidiasis of the hard palate is evident, Reflection of light is noted off bubbles of saliva (arrow)
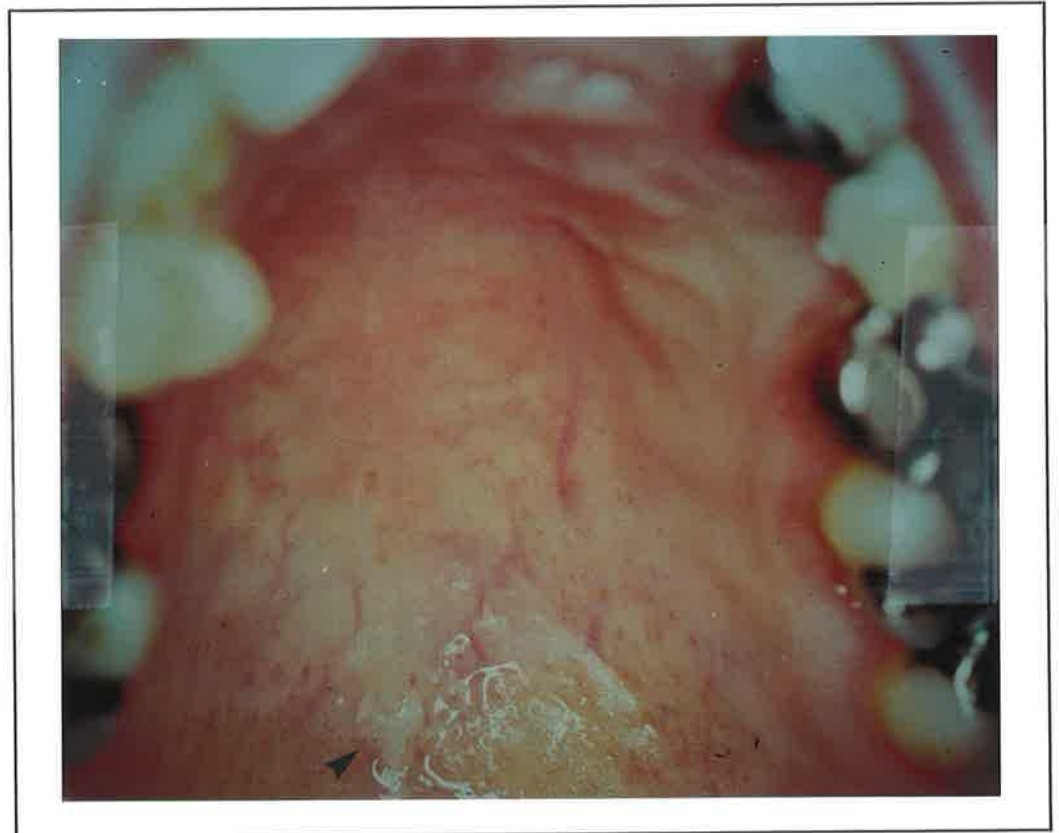
The details of the five patients who showed clinical resolution of their oral candidiasis, but who were withdrawn from the trial because of non-compliance, is shown in Table 5.

Table 6 **Summary of clinical findings in the five patients who despite resolution of their oropharyngeal candidiasis using the non-standard dose of Fungilin® lozenges, were withdrawn from the trial due to non-compliance**

Patient	Oropharyngeal candidiasis
4	After 2 weeks of 8 lozenges daily patient 4 had no evidence of soft palate pseudomembranous candidiasis and symptoms of burning retro-sternally also improved. He refused to dissolve the lozenges orally without his full upper denture present. This prevented the lozenges from acting on the hard palate and resolving the pseudomembranous candidiasis beneath the full denture.
8	Resolution of pseudomembranous candidiasis on tonsillar fossae after one week. Patient failed to attend further reviews after 2 weeks into the trial and was withdrawn for non-compliance.
11	Resolution of all clinical signs of oropharyngeal candidiasis after the first week of 8 lozenges daily. Patient ceased taking lozenges in week 7 for one week. He resumed lozenges for a period of two weeks with marked reduction in the extent of oropharyngeal candidiasis, which however still persisted. Patient was withdrawn at week 9 and placed on fluconazole.
13	Resolution of pseudomembranous and erythematous candidiasis at the non-standard dosage, but patient failed to attend further follow-up reviews. Patient was withdrawn due to non-compliance.
19	Resolution of angular cheilitis and pseudomembranous candidiasis of the tongue and buccal mucosa within the first week. Patient was withdrawn because of failure to attend further review appointments.

4.2.2 Culture results from oral rinses taken at time of trial entry

At the commencement of the trial nineteen of the twenty patients had *C. albicans* cultured from their oral rinses (Figure 18). In addition patient 3 had a non-albicans species cultured. The oral bacterial status of all twenty patients was consistent with normal oral flora. However, patient 4 had a positive culture for *Pseudomonas aeruginosa*. The number of *C. albicans* colonies grown on an agar plate varied between patients. Results are presented in Table 7.

Figure 18 Graphical representation of the proportion of *Candida* cultured from the oral rinses of all 20 patients at the commencement of the trial
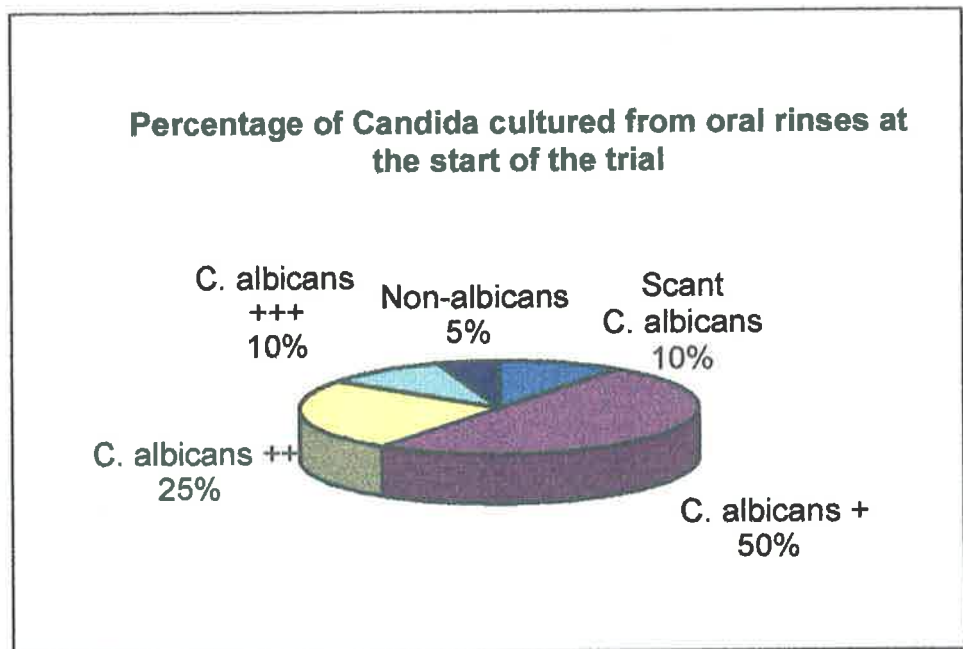
Percentage of Candida cultured from oral rinses at the start of the trial

C. albicans +++ 10%

Non-albicans 5%

Scant C. albicans 10%

C. albicans ++ 25%

C. albicans + 50%

Legend	
Scant C. albicans	Represents a few (1-2) colonies on culture
C. albicans +	Represen pts less than 10 colonies on culture
C. albicans ++	Represents 10-20 colonies on culture
C. albicans +++	Represents greater than 30 colonies of C. albicans on culture

Table 7 **The species of yeast cultured from the oral rinse of each patient at the commencement of the trial**

Patient enrolled	Culture of yeasts at commencement of trial	Microscopic presentation of yeasts at commencement of trial
1	C. albicans +	No yeast cells
2	C. albicans ++	Occasional yeast cells
3	Yeast cells ++ (species to be determined)	Yeast cells with pseudohyphae ++
4	Scant C. albicans + and Pseudo aeruginosa	Occasional yeast cells with pseudohyphae
5	C. albicans +	Yeast cells with pseudohyphae +
6	Scant growth of C. albicans	Occasional yeast cells with pseudohyphae
7	C. albicans +	Occasional yeast cells with pseudohyphae
8	C. albicans +	Yeast cells with pseudohyphae +
9	C. albicans +	Occasional yeast cells
10	C. albicans +++	Yeast cells with pseudohyphae ++
11	C. albicans ++	Yeast cells with pseudohyphae ++
12	C. albicans +++	Yeast cells with pseudohyphae
13	C. albicans +	Occasional yeast cells with pseudohyphae
14	C. albicans +	No yeast cells
15	C. albicans ++	Occasional yeast cells with pseudohyphae
16	C. albicans ++	Yeast cells with pseudohyphae
17	C. albicans +	Yeast cells with pseudohyphae +
18	C. albicans +	No yeast cells
19	C. albicans ++	Occasional yeast cells
20	C. albicans +	Occasional yeast cells with pseudohyphae

4.2.3 Culture results taken from an oral rinse at end of the trial

At 12 weeks those patients who completed the trial had either scant *C. albicans* present within their oral rinse or a non-albicans yeast. Results are summarised in Table 8.

Culture from the last oral rinse taken from the eight patients who withdrew from the trial are also included in Table 8. Six of these eight subjects showed *C. albicans* in similar amounts to those present in their initial oral

rinse. Patient 3 showed a high titre of non-albicans oral yeasts and patient 4 showed scant *C. albicans*.

The percentage distribution of both *Candida* species and other yeasts for all subjects is shown in Figure 19.

Table 8 **The species of yeast cultured from the oral rinse of each patient at the completion of the trial. The symbol * indicates patients who withdrew from the trial**

Patient enrolled	Culture of yeast's at completion of the trial	Microscopic appearance of yeast's at completion of trial
1	No Candida species (to be determined)	No yeast cells
2	Scant C. albicans	No yeast cells
3 *	Yeast cells, not C. albicans ++	Yeast cells with pseudohyphae
4 *	Scant C. albicans	No yeast cells
5	Scant yeast cells, not C. albicans	Occasional yeast cells with pseudohyphae
6	Nil Candida species (to be determined)	No yeast cells
7	Non Candida species	No yeast cells
8 *	C. albicans ++	Yeast cells with pseudohyphae ++
9 *	C. albicans +	Occasional yeast cells
1 0	Scant growth of C. albicans	No yeast cells
1 1 *	C. albicans ++	Occasional yeast cells with pseudohyphae
1 2	Scant growth of C. albicans	No yeast cells
1 3 *	C. albicans +	Yeast cells with pseudohyphae
1 4	No candida species to be determined	No yeast cells
1 5	Scant growth of C. albicans	No yeast cells
1 6 *	C. albicans ++	Unavailable
1 7	Scant growth of C. albicans	Occasional yeast cells
1 8	Scant growth of C. albicans	No yeast cells
1 9 *	C. albicans +	No yeast cells
2 0	Scant growth of C. albicans	No yeast cells

Figure 19 Graphical representation of the proportion of *Candida* cultured from the oral rinses of all 20 patients at completion of the trial

Percentage of Candida cultured from final oral rinse

Non-Candida 20%

Scant C. albicans 40%

Non-albicans 10%

C. albicans ++ 15%

C. albicans + 15%
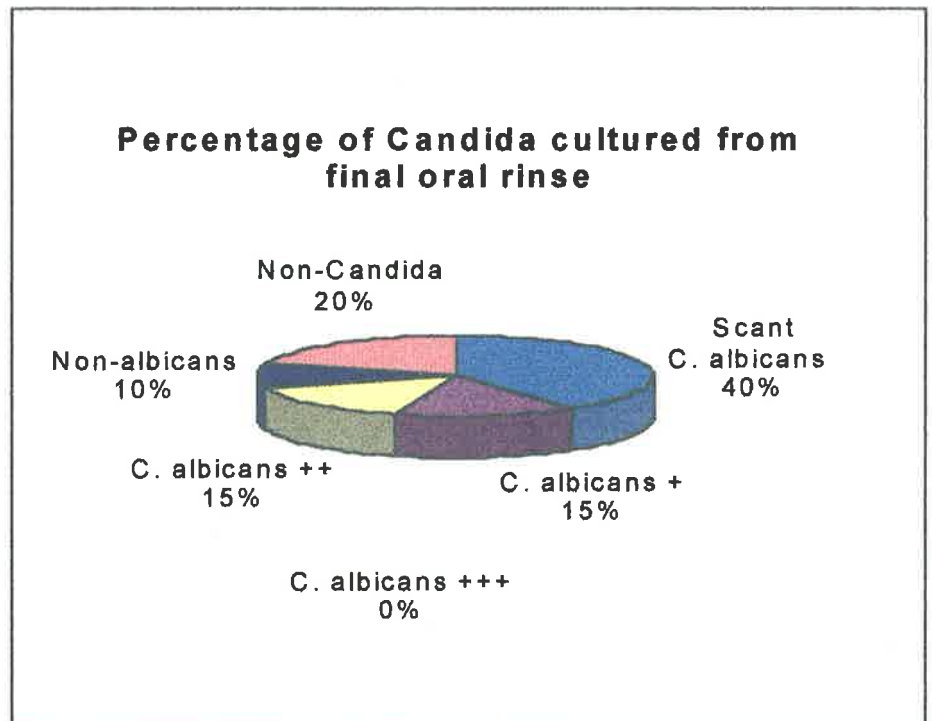
C. albicans +++ 0%

Legend	
Scant C. albicans	Represents a few (1-2) colonies on culture
C. albicans +	Represents less than 10 colonies on culture
C. albicans ++	Represents 10-20 colonies on culture
C. albicans +++	Represents greater than 30 colonies of C.albicans on culture

4.3 HIV Immune Cell Markers

4.3.1 At commencement of the trial

For each patient CD4, CD8 and total lymphocyte counts were recorded at the time of trial entry and at trial completion. These data also included the patients' CD4:CD8 ratio as supplied by the Institute of Medical and Veterinary Science, Adelaide, South Australia. Table 9 summarises each patients peripheral CD4 T-cell lymphocyte count, CD4 and CD8 percentage, CD4:CD8 ratio when available and total lymphocyte cell counts at time of entry into the drug trial. Figures 20 and 21 illustrate the CD4 and CD8 percentages and CD4 and lymphocyte counts for each patient in graphical form.

Data for patient 3 and 17 were unavailable because the patients refused blood tests as part of their medical management. We were unable to locate any blood results for Patient 14, apart from his CD4 cell count at start of the trial.

Table 9 HIV immune cell marker status for each patient at the commencement of the trial. The CD4 cell count and total lymphocyte cell counts are expressed as per uL of blood. The CD4 percentage is determined as a percentage of total lymphocytes expressing the CD4 antigen per uL of blood. Some data were unavailable and is recorded as such

Patient	CD4 cell percentage	CD8 cell percentage	CD4:CD8 ratio	Total Lymphocytes	CD4 cell count
1	10	35	0.29	1845	185
2	28	40	0.76	1892	530
3	Unavailable	Unavailable	Unavailable	Unavailable	Unavailable
4	<1	37	Unavailable	492	<5
5	<1	42	Unavailable	1204	<12
6	24	54	0.44	2102	483
7	19	56	0.34	2572	489
8	12	73	0.16	1648	198
9	9	2	Unavailable	525	47
10	<1	66	0.01	771	<8
11	20	56	0.36	1953	391
12	28	52	0.54	1348	377
13	20	55	0.36	1916	383
14	Unavailable	Unavailable	Unavailable	Unavailable	320
15	3	44	0.07	1189	36
16	2	21	0.1	200	4
17	15	70	Unavailable	Unavailable	450
18	15	64	0.23	2577	387
19	16	64	0.25	2874	460
20	12	72	0.17	1643	197

Figure 20 Each patients CD4 and CD8 cell percentages at the commencement of the trial. Unavailable data has been entered as a zero value in this graph

Figure 21 Each patient's CD4 cell and total lymphocyte cell counts at the commencement of the trial. Unavailable data has been entered as a zero value in this graph

4.3.2 At completion of the trial

The CD4 cell counts, CD4 and CD8 percentages, CD4:CD8 ratio and total lymphocytes data were recorded when each patient had either completed the trial or withdrawn from the trial (Table 10 and Figures 22 and 23). The data represents the most recent blood tests taken from each patient. Results of blood tests from patients 3, 14 and 17 were unavailable because they were not having regular blood tests. Several of the CD4:CD8 ratio results were also unavailable.

Table 10 The HIV immune cell marker status for each patient at the completion of the trial. The CD4 T-cell lymphocyte count and total lymphocyte cell counts are expressed as per uL of blood. The CD4 percentage is determined as a percentage of total lymphocytes expressing the CD4 antigen per uL of blood. The CD4 to CD8 cell ratio is recorded when available. Some data were unavailable

Patients enrolled	CD4 cell count	CD4 cell percentage	CD8 cell percentage	CD4:CD8 ratio	Total lymphocytes
1	379	22	58	0.38	1723
2	593	31	41	0.76	1913
3	Unavailable	Unavailable	Unavailable	Unavailable	Unavailable
4	<5	<1	37	Unavailable	492
5	<10	<1	32	Unavailable	1044
6	759	44	41	1.07	1724
7	315	25	53	0.47	1261
8	198	12	73	0.16	1648
9	44	7	42	0.17	633
10	<8	<1	66	0.01	771
11	145	12	54	0.22	1208
12	390	31	53	0.58	1258
13	383	20	55	0.36	1916
14	Unavailable	Unavailable	Unavailable	Unavailable	Unavailable
15	25	3	63	0.05	839
16	4	2	21	0.1	200
17	Unavailable	Unavailable	Unavailable	Unavailable	Unavailable
18	292	16	63	0.25	1826
19	460	16	64	0.25	2874
20	234	13	70	0.19	1803

Figure 22 Each patient's CD4 and CD8 cell percentages at the completion of the trial. Unavailable data has been entered as a zero value
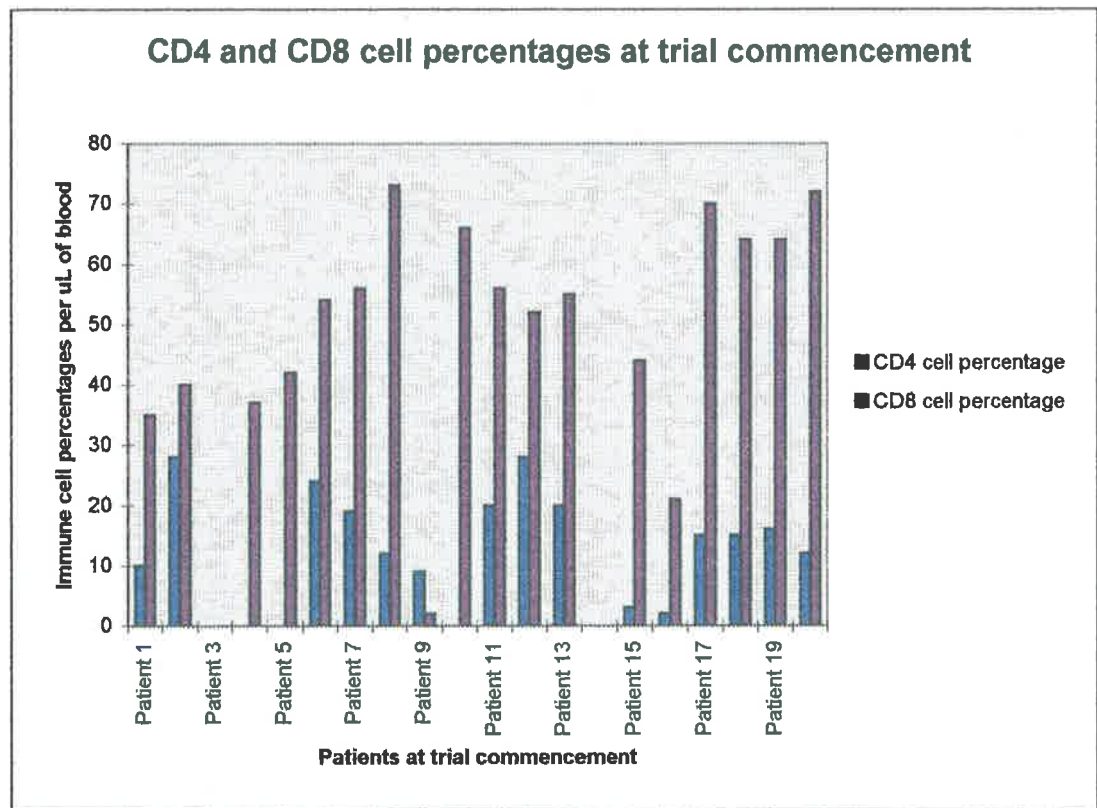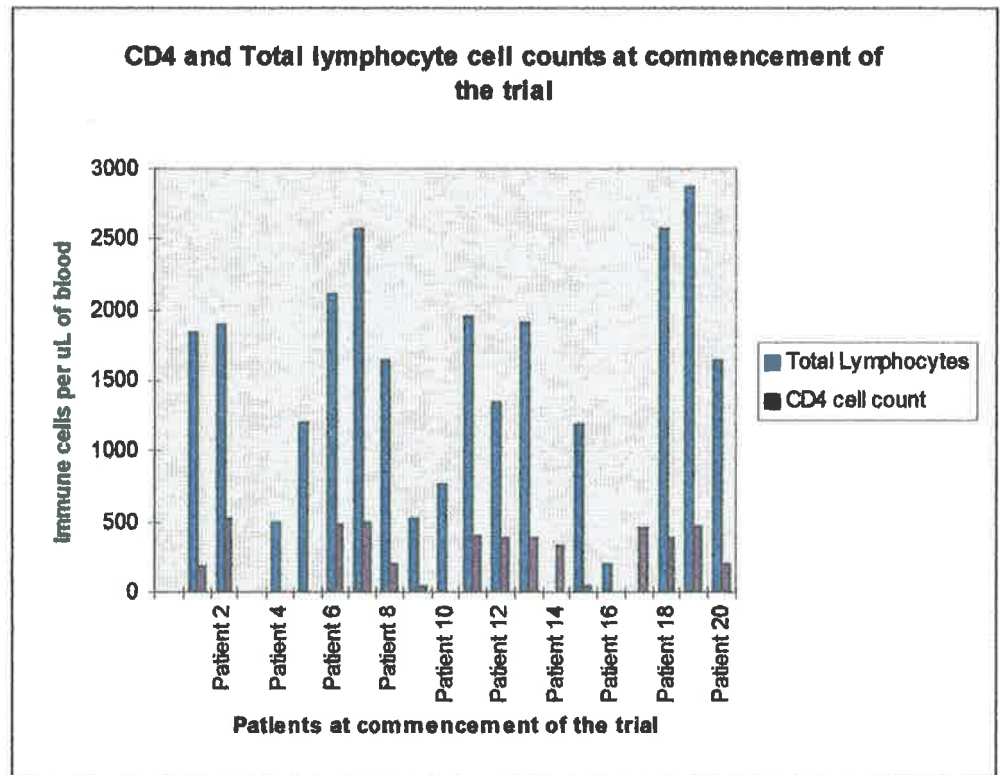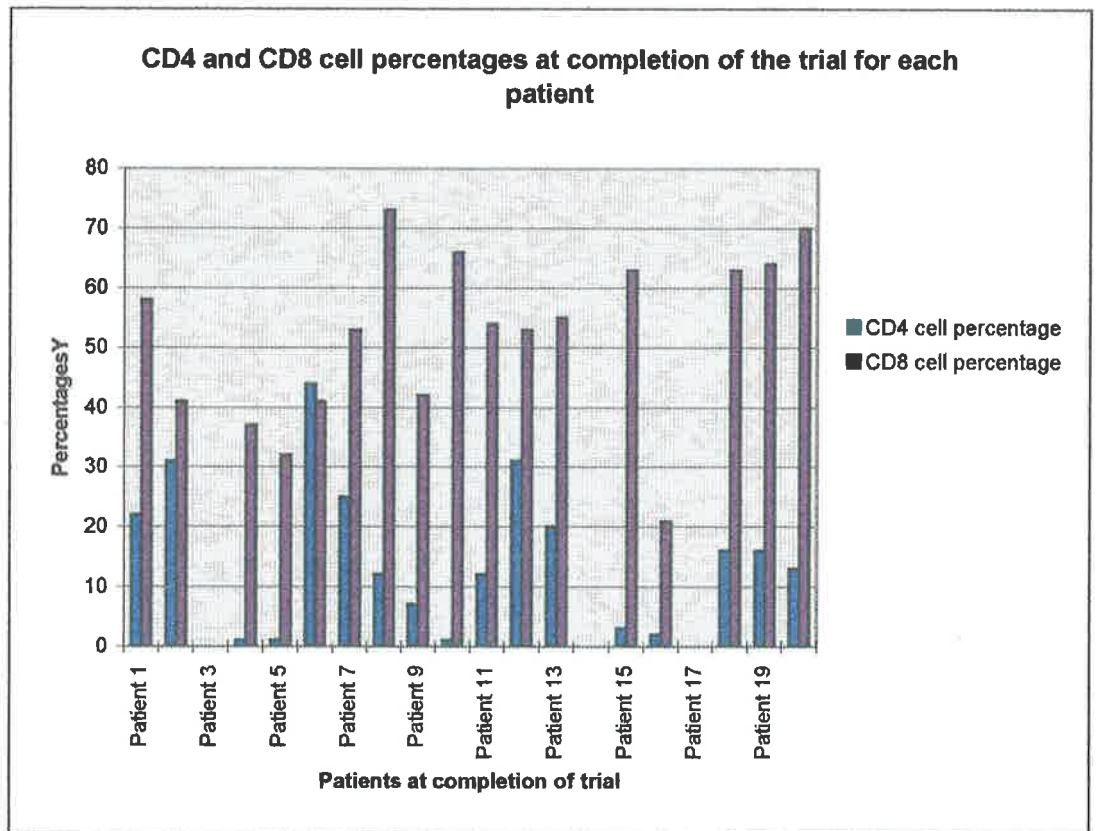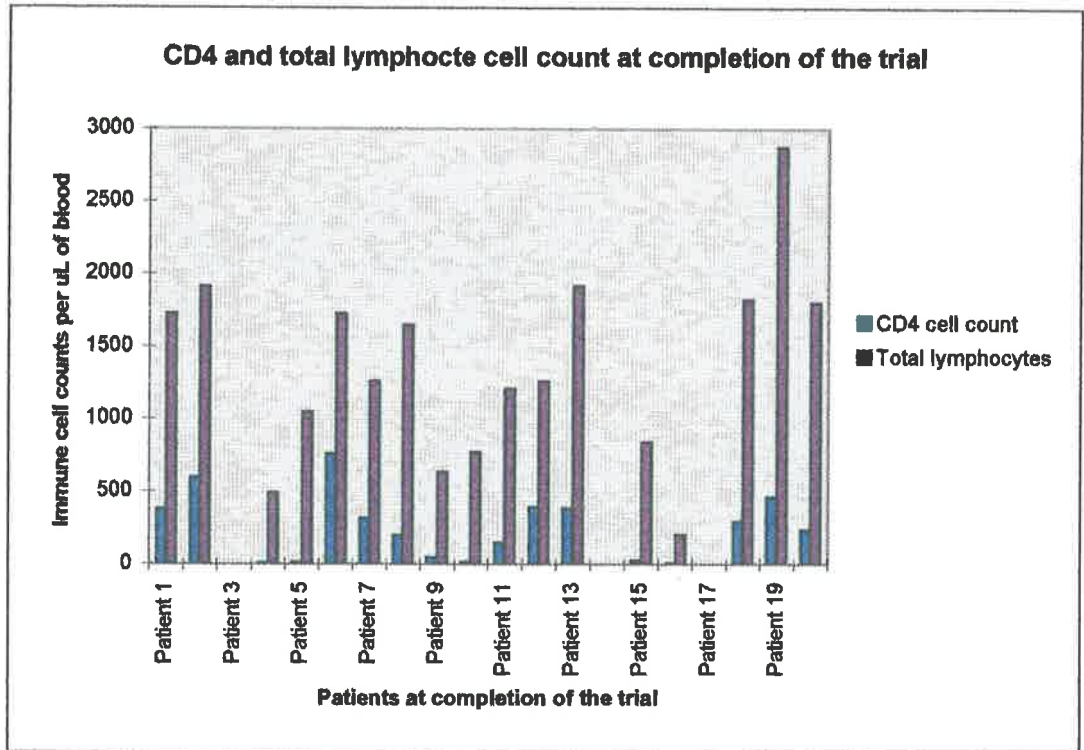
Figure 23 Each patient' s CD4 cell and total lymphocyte cell counts at the completion of the trial. Unavailable data has been entered as a zero value

4.3.3 Comparison of data between trial commencement and completion

Using the previous data, a comparison of the CD4 and CD8 T-cell lymphocyte percentages, and the CD4 and total lymphocyte cell counts between trial commencement and completion was undertaken as shown in Table 11. Of the 12 patients that completed the trial, patient 10 had no change in any of his immune cell markers. Patients 17 and 14 data was unavailable. The remaining 9 patients that completed the trial had changes recorded in most or all of the immune cell markers. In addition, of the 8 patients that were withdrawn from the trial only patients 9 and 11 had recorded changes in their immune cell markers. Patient 3 data was unavailable.

Table 11 Comparison of HIV immune cell markers for each patient between commencement and completion of the trial. An increase is expressed as a 'positive' sign, and a decrease as a 'negative' sign. If there was no change in the data available between trial commencement and completion, then this is expressed as 'no change' for each category. An * indicates patients who were withdrawn/withdrew from the trial

Patients	CD4 percentage	CD4 cell count	CD8 percentage	Total lymphocytes	Trial status
1	12+	194+	23+	122 -	Completed
2	3+	63+	1+	21+	Completed
3 *	Unavailable	Unavailable	Unavailable	Unavailable	Withdrew after 3.5 weeks
4 *	No change	No change	No change	No change	Withdrew after 8 weeks
5	No change	2 -	10 -	160 -	Completed
6	20+	276+	13 -	288 -	Completed
7	5+	174 -	3 -	1200 -	Completed
8 *	No change	No change	No change	No change	Withdrew after 2 weeks
9 *	2 -	3 -	40+	110+	Withdrew after 4 weeks
10	No change	No change	No change	No change	Completed
11 *	8 -	246 -	2 -	745 -	Withdrew after 9 weeks
12	3+	13+	1+	No change	Completed
13 *	No change	No change	No change	No change	Withdrew after 1 week
14	Unavailable	Unavailable	Unavailable	Unavailable	Completed
15	No change	11 -	19+	350 -	Completed
16 *	No change	No change	No change	No change	Withdrew after 1 week
17	Unavailable	Unavailable	Unavailable	Unavailable	Completed
18	1+	95 -	1 -	751 -	Completed
19 *	No change	No change	No change	No change	Withdrew after 1 week
20	1+	37+	2 -	160+	Completed

4.4 Medications That Changed During The Trial

An important variable during the trial was the difference in medications taken by each patient. Furthermore, six patients changed their medication during the trial (Table 12).

Table 12   **Summary of medication status of all twenty patients enrolled in the trial. If no change in any of the patients' medications occurred while enrolled in the trial, then this was recorded as 'no change'**

Patient	Medication(s) that were changed during the drug trial
1	Addition of nucleoside analogue d4T
2	Commenced triple combination therapy, was on no medication at time of drug trial entry AZT, d4T and 3TC
3	No change in medication
4	No change in medication
5	Ceased Clarithromycin and Ethambutol
6	Commenced Rulide, and 2 nucleoside analogues AZT and 3TC
7	No change in medication
8	No change in medication
9	Commenced Fluconazole, Acyclovir
10	Changed nucleoside analogue to 3TC
11	No change in medication
12	No change in medication
13	No change in medication
14	No change in medication
15	No change in medication
16	No change in medication
17	No change in medication
18	No change in medication
19	No change in medication
20	No change in medication

4.5 Amphotericin B Sensitivity Testing

The *C. albicans* cultured from the oral rinses taken at the commencement and completion of the trial for each patient were tested for sensitivity to amphotericin B. Table 13 presents the results of sensitivity testing to amphotericin B.

Table 13   Table showing results of amphotericin B sensitivity at beginning and end of the trial. Subjects who were withdrawn/withdrew before the 12 weeks are designated with an *

Patient	Sensitivity or resistance to amphotericin B at the start of the trial	Sensitivity or resistance to amphotericin B at the completion of the trial
1	Sensitive	Sensitive
2	Sensitive	Sensitive
3*	Sensitive	Sensitive
4*	Sensitive	Sensitive
5	Sensitive	Sensitive
6	Sensitive	Sensitive
7	Sensitive	Sensitive
8*	Sensitive	Sensitive
9*	Sensitive	Sensitive
10	Sensitive	Sensitive
11*	Sensitive	Sensitive
12	Sensitive	Sensitive
13*	Sensitive	Sensitive
14	Sensitive	Sensitive
15	Sensitive	Sensitive
16*	Sensitive	Sensitive
17	Sensitive	Sensitive
18	Sensitive	Sensitive
19*	Sensitive	Sensitive
20	Sensitive	Sensitive

4.6 Statistical Analysis

The statistical analysis of applicable trial results was undertaken by the Department of Statistics, The University of Adelaide using BMDP Statistical Software, UCLA, United States of America. (Program 4F)

For each review session (weeks 1, 2, 4, 8 and 12) each patient's results were evaluated according to two categories. The first sub-category was in the 'Observed' effect, based on the clinical evidence of oropharyngeal candidiasis being present or absent. The second sub-category was in the Culture result, whether *C.albicans* was present in each oral rinse taken.

According to Kauffman et al (1986) and Fotos et al (1992) cytologic evidence of fungal pseudohyphae occurring simultaneously with clinical signs and symptoms remains the primary basis for the diagnosis of oral candidiasis. Hence, each patient was considered a 'successful' outcome if they had no clinical signs and symptoms, and no *C. albicans* cultured from their oral rinses. Patients were also considered as having a 'successful' outcome if either one of the clinical or mycology sub-category variables were present, but not both.

A 'failure' result was recorded when both clinical signs and symptoms of oropharyngeal candidiasis and an oral rinse culture positive for *C. albicans* were present. Patients that withdrew from the trial, and did not attend any further review sessions were also categorised as 'failures'.

The trial period of 12 weeks was divided into 2 parts. The first 2 weeks with a daily drug dose of 80mg of amphotericin B, was labelled 'D80'. The remaining 10 weeks with a daily drug dose of 40mg of amphotericin B, was labelled 'D40'.

4.6.1 Overall outcome from the trial data

The statistical analysis questioned whether the overall success (S) and failure (F) rate for all patients enrolled in the trial was statistically significant compared to a 50/50 outcome. The Chi Square value calculated was 0.404, and the critical value for chi square on 1 degree of freedom is 3.84. Hence there was no statistical evidence that the success/failure rate was different from 50%.

4.6.2 The success rate for each patient succeeding the 12 week trial if they completed the initial 2 weeks of 80mg of topical amphotericin B

If a successful result was obtained from each patient at the end of week 2 receiving a topical dose of 80mg of amphotericin B daily, then using a conditional success/failure for the remaining 10 weeks of the trial, the Chi square value was calculated as 4.094. Given the critical chi square value is 3.84, then statistical evidence to support the success/failure rates was different from a 50/50 result.

This can be interpreted to mean that if the patient achieved a successful outcome after week 2 using the non-standard dose of 80 mg of amphotericin B, then there is an 85% chance of success that the patient's oropharyngeal candidiasis will not relapse while continuing to use topical amphotericin B lozenges at the maintenance dose of four lozenges daily.

CHAPTER 5

DISCUSSION

DISCUSSION

5.0 PATIENT SELECTION AND COMPLIANCE

5.1 Subject Selection

A total of twenty patients were enrolled in the trial. The age of the subjects ranged from 24 years to 55 years with a mean age of 39 years and 11 months. Nineteen of the enrolled patients were male homosexuals and one was a bisexual male. One homosexual and one bisexual patient were also intravenous drug users. Many of them were at different stages in their HIV disease.

Figures from the South Australian Health Commission on HIV infection in South Australia (1996) reported that between 1985 and 1995, 602 individuals in South Australia were diagnosed with HIV infection. Ninety three percent of these individuals were males. The most common risk factor for becoming HIV infected was male to male sexual contact in 75% of cases. A further 9% reported an injecting drug use habit and 5% of males reported a history of both risk factors.

These risk profiles were reflected in the subject profiles enrolled in this trial. Nineteen or 95% of the trial participants reportedly acquired HIV infection from male to male sexual contact. The remaining participant acquired HIV infection from intravenous drug use.

5.2 The Variation In HIV Disease Expression Among Subjects Enrolled

Marriott and McMurchie (1997) reported that according to the United States Centres for disease control AIDS was defined by a peripheral CD4 positive T-cell lymphocyte count of < 200 uL, regardless of the patient's clinical condition. According to this definition nine patients enrolled in the drug trial had full blown AIDS. The remaining eleven patients were in the intermediate stage of HIV infection according to the classification proposed by Stewart et al (1996). That is, their CD4 positive T-cell lymphocyte cell count ranged between 500 cells uL and 200 cells uL of blood.

Of the twenty enrolled patients thirteen had an opportunistic infection apart from oropharyngeal candidiasis at commencement of the trial. The most frequently encountered was oral hairy leukoplakia (OHL) found in eight of the twenty patients. Both Melnick et al (1988) and Greenspan and Greenspan (1992), observed that the presence of OHL was an important predictor for the patient to develop AIDS. The median time to develop full blown AIDS when OHL was detected is approximately 24 months and the mean time to death was observed by Greenspan et al (1992) to be 41 months from the development of OHL.

At commencement of the trial, patients 11, 13, and 18 had bilateral OHL with CD4 cell counts of 391, 383 and 387 respectively. The OHL may have been related to a decline of the immune system in these patients which was not truly reflected by the CD4 cell count. This may, in part, explain the dramatic recurrence of oropharyngeal candidiasis seen in patient 11 at week 8 of the trial. The peripheral CD4 positive T-cell lymphocyte count of patient 11 fell from 391 cells per uL at the commencement of the trial to 145 cells per uL at week 8 of the trial. This patient (at week 7) had failed to use the topical amphotericin B (Fungilin[®]) lozenges for seven days. Up until this time his oro-pharynx had remained clinically free of candidiasis.

5.3 Patient Compliance

A review on compliance in drug trials by Besch (1995), noted that most patients take 'drug' holidays or make their own decisions about dose adjustments or timing of the medications. Besch (1995) concluded that this was especially true for short therapeutic courses and when treatment was preventative rather than curative.

This phenomenon was evident in the trial, particularly with patients 4, 8, 9, 11, 13 and 19. Of these, patients 4, 9 and 11 were non-compliant by failing to use the topical amphotericin B lozenges as instructed. Patient 4 refused to remove his full upper denture to enable exposure of the hard palatal mucosa to come into contact with the lozenges. He then failed to attend his review session at week 8. Patient 9 was failing to suck the lozenges daily, despite reporting that he was. Because of failure to resolve the initial signs of oropharyngeal candidiasis, and as the C. *albicans* cultured from his oral cavity was sensitive to amphotericin B, it was concluded that he was non-compliant and was withdrawn from the trial. Patient 11 had ceased taking the Fungilin® lozenges for seven days.

The other three patients (8,13 and 19) were withdrawn from the trial because they failed to attend follow-up review appointments. Once their oropharyngeal candidiasis had resolved they apparently lost interest in the trial and failed to attend review appointments.

Besch (1995) reported that patient compliance in a clinical drug trial could be measured either directly or indirectly. A direct means of measuring compliance was assessment of attendance at review visits. In this trial, five patients were withdrawn because of failure to attend review appointments. This was interpreted as lack of patient compliance.

Regulation of patient compliance in relation to taking each lozenge was difficult. In particular, between the recall visits at weeks 4, 8 and 12, it was impossible to ensure that each patient was sucking four lozenges of Fungilin[®] daily. To review each patient weekly while taking the standard dose of four lozenges daily was considered an excessive number of appointments for each trial subject to attend as many of these patients already had extensive appointment schedules related to the overall management of their HIV disease. At each recall visit, all patients were asked how many lozenges they had remaining. Most patients admitted forgetting to take all of the lozenges daily, particularly when required to use eight Fungilin[®] lozenges per day. Yet despite this, seventeen of the twenty patients at the end of week 1 had clinical resolution of their oropharyngeal candidiasis.

Many of the participants enrolled in the trial commented that it was difficult to remember to take the lozenges throughout the day. In particular during the first two weeks where eight lozenges daily were required. The frequency of using the topical amphotericin B lozenges, rather than the dose used, was fundamental in exposing the *Candida* regularly to the 10 mg dose of topical amphotericin B.

In this study, the length of time that each patient sucked a lozenge was restricted to a standardised minimum of 15 minutes. This was to limit the variability in length of time each dose taken had contact with the oral mucosa. In contrast, immune competent patients enrolled in a study by deVries-Hospers and van der Waaji (1980), using topical amphotericin B in a lozenge form, were required to suck a single lozenge for up to two hours. These investigators found that this maintained a constant therapeutic dose level in saliva to resolve oropharyngeal candidiasis in an immunocompetent patient.

In the present study indirect patient compliance was assessed using several

methods. The first was by assessing the resolution of clinical signs of the oropharyngeal candidiasis. Twelve patients were clinically free of oropharyngeal candidiasis throughout the twelve weeks of the trial. Five patients who were withdrawn from the trial because of non-compliance had resolution of their oropharyngeal candidiasis at the end of the first week of therapy. The remaining three patients (3, 16 and 9) from the total eight patients withdrawn from the trial had no resolution of their oropharyngeal candidiasis at the end of week 1. This was due to the fact that patients 3 and 16 had xerostomia and were therefore unable to adequately suck the lozenges. Patient 9 experienced no resolution of their oropharyngeal candidiasis because of failure to take the Fungilin® lozenges as prescribed.

5.4 Questionnaire In Evaluating Subjects' Views Of The Trial

Patient compliance was also measured indirectly by assessing the questionnaires compiled for each patient during their recall visits. The questionnaire consisted of four questions that were designed to subjectively evaluate the patient's progress during the topical amphotericin B (Fungilin®) trial.

Question one: *How does your mouth feel?* Only patient 12 reported no improvement consistently throughout the trial. This was because he had no symptoms or discomfort from their oral candidiasis at time of the trial onset. The oral candidiasis in patient 12 was initially found during a routine oral examination. Patient 8 reported no improvement at week 1, but did feel improvement after the second week.

Patients 9 and 11 initially reported improvement but changed their opinion later in the trial. Patient 9 during the first two weeks of the trial reported improvement, despite having the presence of extensive oropharyngeal candidiasis from week 1. At

week 4 he changed his opinion and reported no improvement. It was felt from the first review session that lack of compliance was the underlying reason for persistence of the oropharyngeal candidiasis in patient 9. As already indicated cultures of *C. albicans* taken from the oral cavity of patient 9 at the commencement of the trial and at the time of withdrawal from the trial were sensitive to amphotericin B.

Patient 11 reported no improvement at week 8 of the trial following a recurrence of oropharyngeal candidiasis. Until then he had reported improvement in the symptoms of oral discomfort and in the feel of his mouth.

Question two: *Any problems with the medication?* The majority of patients who completed the 12 weeks trial reported no problems with using the lozenges. However, patients 1, 7 and 15 said 'yes ' after the first week of the trial. Patients 1 and 7 reported a side-effect of diarrhoea, and patient 15 reported nausea. These patients attributed the symptoms to side-effects of the amphotericin B lozenges.

At week 2 of the trial, patients 10 and 18 reported having problems with the medication. However, those patients reporting nausea and diarrhoea after week 1 were having no further problems. Patient 10 replied 'yes' because he was having trouble remembering to take all eight Fungilin® lozenges daily. Patient 18 reported some nausea and diarrhoea.

At the end of week 2, no further 'yes' replies to question two were reported by any of the patients that completed the trial.

Three of the eight patients who were withdrawn from the trial replied 'yes' to question two. Patients 16 and 3 replied 'yes' because both were xerostomic and had

difficulty dissolving the lozenges intra-orally. In week 2, patient 8 said he felt 'bloated' and attributed this to the Fungilin® lozenges.

The remaining 'yes' responses during the last ten weeks of the trial, were comments regarding difficulty in remembering when to take the lozenges daily.

The side-effects of topical amphotericin B reported from Goodman et al (1996) are occasional nausea and diarrhoea, unlike the severe nephrotoxicity and azotemia as seen in systemic administration of amphotericin B. This is because of the negligible absorption of topical amphotericin B from the gastrointestinal tract. A problem in this trial was differentiating the reported nausea and diarrhoea symptoms from patients 1, 7, 15 and 18 as being either directly related to the use of topical amphotericin B, caused by other medications the patient was taking at the time of the trial, or associated with their HIV disease. Because of the short duration of nausea and diarrhoea for each of the patients it would appear that their symptoms were not related to the use of topical amphotericin B (Fungilin®) lozenges.

Question three: *Any change in taste?* Most of the enrolled patients reported no change in taste, associated with the use of the Fungilin® lozenges. Those patients who did reply 'yes' to this question described the change in taste as an improvement. Patients 11, 18 and 2 described their tongues as feeling less 'furry'. Only patient 4 commented that his taste of milk felt slightly different.

None of the twenty patients enrolled in the topical amphotericin B trial complained about any unpleasant taste associated with the lozenges. This is in contrast to what is often reported in the literature. For example, Cartledge (1993) concluded that the poor taste associated with many of the topical antifungal agents is often why the use of topical antifungal agents are ineffective as a treatment modality for use in the resolution of oropharyngeal candidiasis within the HIV population.

Question four:*Any improvement in the symptoms since commencing the trial?*. This question was asked to assess whether the patient felt they were getting benefit from being enrolled in the trial. Patients 7 and 12 reported no improvement throughout the trial as they had no symptoms or discomfort from their oral cavity at commencement of the trial. Patients 16 and 3 reported no improvement in their symptoms, because of xerostomia. Presumably because they were unable to suck the lozenges no therapeutic benefit from the amphotericin B resulted.

5.5 Trial Design

5.5.1 Problems encountered in establishing the trial

A major problem in establishing this trial was a lack of patient participation. Many of the HIV positive patients contacted were concerned about the side-effects associated with the use of topical amphotericin B. In particular, misinformation within the HIV positive community about the risks of nephrotoxicity and bone marrow suppression was a significant problem. Many potential subjects thought that these side effects also occurred from using the topical form. This problem ultimately contributed to a small number of enrolments in the trial.

Many of the HIV positive patients initially approached were already on extensive numbers of medications. The additional demands from an electively enrolled drug trial such as remembering to take eight and then later four lozenges daily, was felt by many patients to be too big an imposition.

Originally the trial was to have three groups of twenty HIV positive

patients. The control group was to consist of patients with oropharyngeal candidiasis who had not been prescribed any antifungal medication for at least three months prior to enrolment in the trial. The control group was to be prescribed the non-standard dose of eight lozenges daily for two weeks, followed by four lozenges daily for the remaining ten weeks.

The remaining two groups were to be made up of HIV positive patients who had fluconazole resistant oropharyngeal candidiasis. These patients were to be randomly assigned to either subgroup. The first subgroup were to be prescribed eight lozenges daily for the first two weeks, followed by four lozenges daily for the remaining ten weeks. The second subgroup was to be prescribed four lozenges daily for the entire twelve weeks.

The original aim of this study was to demonstrate the effectiveness of using topical amphotericin B (Fungilin®) at a non-standard dose of eight lozenges daily in both the control and fluconazole resistant oropharyngeal candidiasis subgroups. This was to assess if using a non-standard dose was effective in resolving clinical signs and symptoms of oropharyngeal candidiasis in either or both groups.

The remaining fluconazole resistant oropharyngeal candidiasis subgroup was to be given four lozenges daily for the twelve weeks was to compare the efficacy of the standard dose of topical amphotericin B (Fungilin®) in comparison to the other fluconazole resistant subgroup on the higher dose. A personal clinical observation prior to setting up this trial found that using a greater than standard dose of four lozenges daily could resolve the oropharyngeal candidiasis in HIV positive patients.

The problem of fluconazole resistant *Candida* is often reported in the

literature. Diz Dios et al (1994) concluded that since 1992 the emergence of fluconazole resistant oral candidiasis was becoming a more frequent problem. As already indicated an original aim of this trial was to assess the effectiveness of using topical amphotericin B in resolving any signs and symptoms of fluconazole resistant oral candidiasis.

5.5.2 Areas of the trial that could have improved the outcome

Using topical amphotericin B in a gel form rather than as a lozenge may have enabled five of the patients enrolled in the trial who suffered varying degrees of xerostomia to use the medication more effectively.

Patients 3 and 16 were xerostomic because of intra-oral radiotherapy associated with treatment for intra-oral lymphoma and Kaposi's sarcoma respectively. Xerostomia in partients 14 and 10 was a result of the daily use of methadone.

The xerostomia associated with patient 9 was thought to be directly related to his HIV infection. The incidence of xerostomia in HIV disease is high, and may occur because of salivary gland enlargement observed in the parotid salivary glands. Greenspan et al (1990) concluded that xerostomia may occur in 10-13 percent of patients with AIDS. Greenspan also concluded that there is no difference in the average salivary flow rate between groups of patients with and without AIDS, there are some individual patients with Aids Related Complex and AIDS who do suffer from a severely reduced salivary flow rate.

Currently we are awaiting results from the 'DNA fingerprinting technique', to evaluate if any of the *C. albicans* species detected in the oral

rinses from subjects had undergone any phenotypic alterations. This technique will also enable identification of non *Candida* species found in patients 1, 3,6, and 14.

The sampling of oral *Candida* species present in the oral cavities of the patients' sexual partners would have been useful. Several studies such as those by Boerlin et al (1995) and Heald et al (1996) demonstrated that transmission of *C. albicans* can occur between sexual partners. The assessment of both the genotype and phenotype of *Candida* in the trial subjects and their sexual partners is a potentially important variable that may explain why *C. albicans* wasn't completely cleared from their oral cavities. It would also have been useful to assess if the non-*Candida* yeasts sampled from the oral cavity were endogenous to the enrolled patient, or acquired.

5.6 The Effect Of High Dose Topical Amphotericin B In The Study

5.6.1 Side-effects reported

Of the twenty patients enrolled in the study, four patients reported nausea or diarrhoea for a short time during the trial. In the first week, patients 7 and 1 reported diarrhoea. Patient 15 reported nausea. Despite this, all three patients continued with the trial and reported no further discomfort. Patient 18 reported some nausea and diarrhoea in week 2 of the trial. Again, he continued with resolution of those symptoms. These symptoms may have been related to the higher dose of eight lozenges daily. However, given that they were only taken for a short duration it would seem reasonable to suggest that these symptoms were unrelated to taking the Fungilin[®] lozenges. Patients 7 and 18 were on anti-retroviral drugs AZT and ddC

5.7 Patient Variables That May Have Affected The Outcome

5.7.1 CD4/CD8 relationships

Both Carpenter et al (1990) and McCarthy (1992), reported that the incidence of oropharyngeal candidiasis in a HIV positive patient most likely occurred at a CD4 positive T-cell lymphocyte count of less than 300 cells per uL of blood. The development of oesophageal candidiasis was most likely to occur with a CD4 positive T-cell lymphocyte count of less than 100 cells per uL of blood. The CD4 positive T-cell lymphocyte count of subjects at entry into the trial ranged from 530 to 4 cells per uL of blood, with a mean CD4 positive T-cell lymphocyte count of 261 cells per uL of blood.

In the twelve patients that completed the trial, nine of these had altered CD4, CD8 and total lymphocyte parameters at some time during the amphotericin B trial. However, patient 10 had no detectable change throughout the trial. The CD4, CD8 and total lymphocyte markers were unavailable for patients 14 and 17.

Patients 1, 2 and 6 during the trial period of twelve weeks had an increase in their CD4 positive T-cell lymphocyte count and percentage. They also had their anti-viral medication changed during the trial period. A possible causal association may be inferred from this change of anti-viral medication and its impact on the patient's immune cell markers.

Patient 1 had a nucleoside analogue d4T added to his medications. Patient 2 at commencement of the trial was not prescribed any anti-viral

medication. He started triple combination therapy of AZT, d4T and 3TC during the trial period. Patient 6 commenced two nucleoside analogues during the trial, AZT and 3TC. Patients 7 and 18 had a dramatic decline in their CD4 cell number and total lymphocyte cell count during the trial period. Patient 7 had a decline in his CD4 count of 64% and an overall fall in the total lymphocyte count of 49%. Also patient 18 reported no change in any anti-viral medications yet a reduced CD4 count of 75% and a fall in his total lymphocyte count by approximately 71% occurred. Despite both of these scenarios patients 1, 2, 6, 7 and 18 did not relapse and develop oropharyngeal candidiasis

Patient 4 also had no recorded change in any of the CD4 cell and CD8 cell parameters and was only withdrawn after 8 weeks because of non-compliance. He also had no medication change. Patient 11 had a radical decline in the immune cell markers, evident at time of being withdrawn from the trial. There was a fall in his CD4 cell percentage, from 20 to 12 percent. Overall his CD4 cell count had fallen 37% and his total lymphocyte count had lowered by approximately 62% since commencing the trial. Patient 11 was withdrawn from the trial because of a recurrence in oropharyngeal candidiasis, which was assumed to have relapsed because of failure to take the amphotericin B (Fungilin[®]) lozenges during week 7. After seven days of non-compliance he recommenced the lozenges in week 8 at a dose of four a day. The recurrence of oropharyngeal candidiasis had only partially improved. It was for this reason only, the patient was withdrawn.

The question remains whether the significant relapse of oropharyngeal candidiasis in patient 11 was due to him failing to maintain the dose of amphotericin B (Fungilin[®]) lozenges daily for those seven days or

whether it was a result of a combination of factors such as the decline of his immune system. At the time patient 11 was withdrawn from the trial the yeast cultured from his oropharyngeal candidiasis was *C. albicans* and was shown to be sensitive to amphotericin B.

After the first week seventeen of the trial patients had clinical resolution of their oropharyngeal candidiasis. This was despite eight of the patients having full blown AIDS. Of these patients 4, 5 and 10 had a CD4 T-cell lymphocyte count less than 12 cells per uL of blood. This finding is contrary to those opinions expressed in several papers (Coleman 1993; Smith and Croser 1991 and Epstein 1990). These investigators concluded that topical amphotericin B lozenges and nystatin suspension are only effective at early stages in HIV disease and are associated with a poorer response in the later stages of HIV disease.

Further, patients 1, 15 and 20 had CD4 positive T-cell lymphocyte counts of 185, 36 and 197 cells per uL of blood respectively. All three patients had resolution of their oropharyngeal candidiasis after the first week, and were maintained clinically free of oropharyngeal candidiasis throughout the twelve weeks of the drug trial.

5.7.2 Medications

The range and combination of medications that each patient was prescribed varied between patients. Analysis of the medication's role, if any, in compounding the effects of resolution of oropharyngeal candidiasis with topical amphotericin B would require a larger sample of trial participants.

5.7.3 Role of co-factors

As already stated, at commencement of the trial subjects had a CD4 positive T-cell lymphocyte counts ranging from 530 to 4 cells per uL of blood with a mean CD4 positive T-cell lymphocyte count of 261 cells per uL of blood.

Ten patients had a CD4 cell count greater than 300 cells per uL of blood yet had both clinical and microbiological evidence of oropharyngeal candidiasis according to the EC-Clearinghouse classification (1992). The likelihood of other factors that may have contributed to the presence of oral candidiasis. This needs to be considered.

Some of these additional co-factors in the development of oropharyngeal candidiasis in HIV positive patients were reported by McCarthy (1992). These co-factors, in addition to xerostomia, included cigarette smoking and whether the patient wore a denture.

Cigarette smoking was reported in nineteen of the twenty patients enrolled. The number of cigarettes smoked ranged from eight cigarettes to a full packet per day. Smoking of marijuana was reported by four patients.

The role of cigarette smoking as a predisposing factor in the incidence of oropharyngeal candidiasis is controversial. Certainly, in this study, 95% of the subjects enrolled did smoke cigarette on a daily basis. In this context a study by Arendorf et al (1980) observed an increase in the numbers of intra-oral *Candida* organisms within the oral cavity of smokers compared to non-smokers. Furthermore McCarthy (1992) reported that tobacco smoking may facilitate the invasion of oral epithelium by *Candida* species,

and that tobacco use has been associated with a reduction in salivary Ig A. However, in a study by McCarthy et al (1991) of risk co-factors for the development of oropharyngeal candidiasis in HIV positive patients the investigators could not demonstrate any statistical significance between cigarette smoking, alcohol consumption and the development of oropharyngeal candidiasis.

In immunocompetent patients Budtz-Jorgensen (1990) and Samaranayake (1990) observed the use of dentures was associated with oral candidiasis and increased carriage of *Candida* species. Three of the patients in the present study had an intra-oral prosthesis and only patient 17 reported that he soaked his dentures in a diluted solution of sodium hypochlorite once a week. His dentures were not swabbed to check the presence of any oral *Candida* organisms and so may still have been a reservoir for *Candida*.

5.8 Candida Data From The Study

Both Kaufman and Jones (1986) and Fotos et al (1992) have demonstrated that in 60% of healthy immune competent patients *Candida* organisms can be cultured from their oral cavities. The controversy within the literature is that no definitive count of these yeasts has been established to allow for differentiation between commensalism and disease. For this reason, cytologic evidence of tissue invasion occurring simultaneously with clinical signs and symptoms remains the primary basis for diagnosing oropharyngeal candidiasis.

The criteria for selection of patients to be enrolled in this trial was based on both a clinical diagnosis of oropharyngeal candidiasis according to the EC-Clearinghouse classification and a positive mycology report from an oral rinse. At commencement of the trial all twenty patients enrolled had clinical signs of oral candidiasis. Of

these, nineteen patients had *C. albicans* cultured from their oral rinse. Only patient 3 had yeast cells detected that were of a non-albicans *Candida* species. This is consistent with the finding of Franker et al (1990) who reported that *C. albicans* was the most frequent yeast cultured from HIV positive oral candidiasis.

The final oral rinse taken from each participant illustrated a variation in the extent and types of *Candida* organisms found in the oral cavity. Of the twelve patients that completed the trial, patients 2, 7, 10, 12, 15, 17, 18 and 20 had scant growth of *C. albicans* cultured in their final oral rinse compared to a range from *C. albicans* + to *C. albicans* +++ cultured from their initial oral rinse.

In addition, patients 1, 5, 6 and 14 had yeasts that were not *Candida* species. There are two possible explanations for the presence of non-*Candida* yeasts. Firstly, this could be due to the emergence of a co-existent yeast species within the oral cavity that was not responsive to the topical amphotericin B (Fungilin[®]) lozenges dose used in the trial. This explanation is consistent with a study by Sullivan et al (1993) who observed that anti-fungal therapy often encouraged the growth of less frequently found *Candida* species. Sullivan et al concluded, that in the immunocompromised patient many different yeast species can exist within the same lesion.

A second possible explanation could be related to transmission or innoculation of yeast organisms from either different sites within the same patient or from a sexual partner. Sangeorzan et al (1994) demonstrated that patients with vaginal candidiasis had similar *Candida* species in other sites of their body. Sangeorzan et al reported that relapses in vaginal candidiasis was possibly due to re-innoculation from distal body sites. A study by Boerlin et al (1995) demonstrated the transmission of candidal strains between HIV sexual partners. As reported earlier in this study it was not possible to have an oral rinse from any of the sexual partners associated

with each enrolled patient. The impact of this potential source of variation thus could not be evaluated.

Of the patients who were withdrawn from the trial, patients 3, 8, 13, 16 and 19 had *C. albicans* cultured in a similar quantity between their initial and final oral rinses. Patient 4 was withdrawn after week 8, because of failure to attend review sessions. His initial and final oral rinses contained a scant *C. albicans* culture.

Patient 11 was withdrawn after week 9, because of an extensive relapse in oropharyngeal candidiasis. As already discussed, the patient had ceased taking his topical amphotericin B (Fungilin®) lozenges for a period of 7 days. At commencement of the trial, patient 11 had only scant *C. albicans* cultured at his review sessions during weeks 1 and 2. At week 4 *C. albicans* was also cultured, but in greater quantities. Despite an erythematous region noted on his hard palate no evidence of pseudomembranous candidiasis was evident. At week 7, patient 11 had ceased taking any topical amphotericin B lozenges for seven days. By week 8 he had extensive oropharyngeal pseudomembranous candidiasis. An oral rinse culture confirmed the presence of *C. albicans*. At week 9, despite being keen to continue with the trial, patient 11 still had both extensive pseudomembranous oropharyngeal candidiasis and a heavy loading of *C. albicans* cultured from the oral rinse. Patient 11 was accordingly withdrawn from the trial, and commenced systemic azole therapy.

CHAPTER 6

CONCLUSION

CONCLUSION

1. This study demonstrated that irrespective of the immune status of a cohort of HIV positive patients high dose topical amphotericin B was effective in resolving the clinical signs and symptoms of oropharyngeal candidiasis.

2. This resolution occurred by 7 days.

3. A maintenance dose of four lozenges daily prevented relapse of oral candidiasis for the 12 week period of the trial in the 12 patients that completed the trial.

4. Eight patients withdrew or were withdrawn from the trial. Clinical resolution of oropharyngeal candidiasis in 5 of these eight patients occurred by the end of the first week.

5. Current clinical management opinions do not favour the use of topical antifungals for the management of oropharyngeal candidiasis, except in the early stages of HIV infection. However, the results of the present study indicate that short term, high dose topical antifungals may be effective in the management of this condition irrespective of a patient's HIV status.

APPENDICES

APPENDIX I

Primary HIV Infection

Clinical Features of Primary HIV infection from Carr and Boyle (1996)

General

Fever

Pharyngitis

Lymphadenopathy

Arthralgia/Myalgia

Lethargy/Malaise

Anorexia/Weight Loss

Neurological

Headache/Retro-orbital pain

Meningoencephalitis

Peripheral Neuropathy

Radiculopathy

Branchial neuritis

Guillian-Barre syndrome

Cognitive/Affective Impairment

Dermatological

Erythematous maculopapular rash

Rosseola-like rash

Diffuse urticaria

Mucocutaneous ulceration

Desquamation

Alopecia

Gastrointestinal

Oral/oropharyngeal candidiasis

Nausea/Vomiting

Diarrhoea

Respiratory

Cough

APPENDIX II

Chronological Framework For Understanding HIV Disease And Its Management Proposed By Stewart et al (1997).

Immune Deficiency

Early (CD4 > 500) 10 weeks–5 years post-infection

Guillain-Barre syndrome

Chronic demyelinating neuropathy

Idiopathic thrombocytopenia

Reiter's syndrome

Polymyositis

Sjogren's syndrome

Bell's palsy

Intermediate (500 > CD4 > 200) 5–10 years post-infection

Tinea, Onychomycosis

Gingivitis

Seborrhoeic Dermatitis

Molluscum Contagiosum

Herpes Zoster

Tuberculosis

Sinusitis

Advanced (CD4 < 200) 10–13 years post-infection

Oral Candidiasis

Oral Hairy Leukoplakia

Herpes Simplex

Cryptosporidiosis

Pneumocystis carnii pneumonia

Toxoplasmosis

Cryptococcosis

Mycobacterium avium complex

Cytomegalovirus

Kaposi's Sarcoma

Non-Hodgkin's Lymphoma

Cervical Intraepithelial Neoplasia

Primary Central Nervous System Lymphoma

APPENDIX III

Information Sheet

The use of topical Amphotericin B Lozenges (Fungilin®) as a topical antifungal in HIV positive patients

It is important that your mouth and teeth are maintained in a healthy state. This is more so for members of the community who are HIV positive, as even minor dental disease will get worse if your resistance to infection is lowered. Oral thrush of the mouth and throat is common in HIV positive patients and can be uncomfortable.

The purpose of this trial is to see if any benefit can be obtained by using a topical antifungal lozenge as treatment either of first choice, or in combination with the systemic antifungal agent that you may be taking at the moment. You will be supplied with the Fungilin® , lozenges as needed at no charge. The only side effects associated with orally administered Fungilin®, lozenges may be some nausea or diarrhoea. In which case you will need to notify us immediately and stop taking the Fungilin®.

Because this trial involves administrating medication once you have agreed to take part in the trial we will obtain a clearance from your medical practitioner for you to start the amphotericin B lozenges.

At commencement of the trial if you are currently taking a systemic antifungal you will be randomly allocated to one of two groups. The first group will begin the trial by taking 8 x 10 mg lozenges daily and dissolving them in the mouth for a 15 minute period during waking hours for the first two weeks. This will be followed by 4 x 10 mg lozenges daily for the remaining 10 weeks of the trial. The second group will commence the trial by taking 4 x 10 mg

lozenges daily for the entire 12 weeks. You will be informed at the commencement of the trial as to which group you have been assigned.

If you are not currently taking any systemic antifungal agents the trial will commence with 8 x 10 mg lozenges daily. Dissolving them in the mouth for a 15 minute period during waking hours for the first 2 weeks. This will be followed by 4 x 10 mg lozenges for the remaining 10 weeks of the trial.

You will have up to 5 appointments over the 12 week period in order for us to assess your mouth. On your initial visit a swab and an oral rinse of 20 ml distilled water will be taken and sent off for culture. This will allow us to check what types of micro-organisms are residing in your mouth before we begin the trial.

During the course of this trial, additional oral rinses and photographs of each surface in your mouth will be taken.

The data accumulated will be kept under strict security and confidentiality is assured. All data will be analysed in an anonymous way. Your name and other personal information will not be recorded except for the purposes of recalling you to enable us to assess your progress during the trial.

It is important to understand that your participation in the project is voluntary. Your eligibility for dental treatment now and in the future will not be altered by your decision to either participate or not in the trial. You may withdraw from the trial at anytime without prejudice to your future dental treatment.

Dr Michael Stubbs Clinic 1.4	ph: 82239235
Dr David Wilson Oral Pathology	ph: 8303071
Dr R Webb Chairman of the Royal Adelaide Hospital Ethics Committee	ph: 82245355

APPENDIX IV

THE UNIVERSITY OF ADELAIDE

CONSENT FORM

See also Information Sheet attached.

1. I _____ (please print) hereby consent to

    take   part in the research project entitled:

    "The use of Amphotericin B (fungilin)as a topical anti-funga

    for the treatment of oropharyngeal candidiasis in HIV

    infected patients ".
2. I acknowledge that I have read the Information Sheet entitled:

    _____

    _____

3. I have had the project, so far as it affects me, fully explained to my satisfaction by the research worker. My consent is given freely.

4. Although I understand that the purpose of this research project is to improve the quality of medical care, it has also been explained that my involvement may not be of any benefit to me.

5. I have been given the opportunity to have a member of my family or a friend present while the project was explained to me.

6. I have been informed that, while information gained during the study may be published, I will not be identified and my personal results will not be divulged.

7. I understand that I am free to withdraw from the project at any time and that this will not affect medical advice in the management of my health, now or in the future.

8. I am aware that I should retain a copy of this Consent Form, when completed, and the relevant Information Sheet.

SIGNED .... DATE...............................

NAME OF WITNESS.. SIGNED...........................
                        (Please print)
                                                            DATE...............................

I, ...have described to ....
              (Please print)

the nature of the procedures to be carried out. In my opinion she/he understood the explanation.

SIGNED.. DATE...............................

STATUS IN PROJECT...

070:HE:Protocol Info Form
Wednesday, 28 November 1990

APPENDIX V

QUESTIONNAIRE

	WEEKS	1	2	4	8	12

1. How does your mouth feel?

2. Any problems with the medication?

3. Any change in taste?

4. Any improvement in the symptoms
since commencing the trial?

ANSWERS:

IMPROVEMENT   : I

NIL IMPROVEMENT   : N

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