بسم الله الرحمن الرحيم

KETOCONAZOLE PHARMACOKINETICS IN SUDANESE PATIENTS WITH MYCETOMA

A thesis submitted

By

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Dedication

To my Family

My Father,

My Mother,

My Brothers & Sisters

To

Dalia & Mutasim

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INTRODUCTION AND LITERATURE REVIEW

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Abstract

The Plasma concentration of ketoconazole was assessed by estimation of the pharmacokinetic parameters for tow regimen 400 mg and 800 mg in mycetoma patients. The study was carried out in the Mycetoma research center, Soba hospital, Faculty of Medicine at the University of Khartoum. Twelve patients adult were included in this study following fulfillment of the inclusion and exclusion criteria of the study. Open randomized design was performed. Blood samples were collected at specified hours after medication with ketoconazole (400 mg or 800 mg). Concentrations of ketoconazole were measured in Plasma by HPLC method. Non-compartmental pharmacokinetic analysis was performed on the Plasma concentration-time data.

The pharmacokinetic parameters (Mean ± SD) of ketoconazole determined following administration of first and last dose 400 mg regimen were estimated as: $T_{1/2}$ 4.24 ± 1.87h and 8.60 h, C_{max} 9.08 ± 3.96µg/ml and 11.56 µg/ml, T_{max} 2.33 ± 0.52 and 6 h, AUC 62.34 ± 31.03 and 183.07 µg.h/ml, MRT 8.15 ± 1.58 and 14.65 h, CL/F 8.78 ± 6.85 and 1.09 L/h, Vd/F 42.70 ± 14.67 and 13.55 L. For first and last dose 800 mg regimen, the parameters were as follows: $T_{1/2}$ 4.00 ± 0.66 h and 6.83 ± 2.15 h, C_{max} 17.21 ± 7.35 and 18.44 ± 7.53 µg/ml, T_{max} 2.17 ± 0.41 and 2.00 ± 0.82 h, AUC_{∞} 121.56 ± 71.52 and 264.56 ± 136.96 µg.h/ml, MRT 7.86 ± 3.28 and 12.49 ± 5.29 h, CL/F 8.79 ± 5.57 and 3.18 ± 2.48 L/h, Vd/F 49.28 ± 27.11and 36.66 ± 40.14 L.

The study concluded that the Plasma concentration of the tow regimens is highly comparable with that of the published studies. Our results support the concept of a change in pharmacokinetics with chronic dosing. High dose may lead to accumulation of ketoconazole in the body and might lead to toxicity

الملخص العربي

تشمل هذه الأطروحة دراسة التمثيل الدوائي داخل الجسم لعقار الكيتوكونازول. تم تقييم التمثيل الدوائي داخل الجسم لهذا العقار عن طريق مقياس التوافر الحيوي لهذا العقار ومقارنتها بالدراسات المنشورة.

أجريت هذه الدراسة بمركز أبحاث الميستوما مستشفى سوبا –كلية الطب جامعة الخرطوم بمشاركة اثنا عشر من المرضى المصابين بالميستوما بعد تحقيق مقاييس التضمين والاستثناء لهذه الدراسة. اتبعت التجارب الإكلينيكية طريقة الاختيار العشوائي المفتوح. تم اخذ عينات الدم في أوقات محددة بعد اخذ جرعة من العقار.تم قياس الكيتوكونازول في البلازما بواسطة التحليل الكروماتوقرافي (HPLC).

تم حساب حركية الدواء (المتوسط ± الخطاء المعياري الكيتوكونازول 400 مليجرام حبوب للجرعة الأولى و الجرعة الاخيره كالآتي: فترة نصف عمر الدواء تساوي 1.87 ± 4.24 و 8.60 ساعة وأعلى تركيز في البلازما يساوي 3.96 ± 9.08 و 9.11 مايكروجرام/مل والزمن اللازم لملاحظة أعلى تركيز يساوي 0.52 ± 2.33 و 6 ساعة والمساحة تحت المنحنى تساوي 31.03 ± 62.34 و 183.07مايكروجرام.ساعة/مل ومتوسط فترة بقاء الدواء في الجسم يساوي 1.58 ± 8.15 و 14.65 ساعة ومعدل بقاء الدواء في الجسم يساوي 6.85 ± 8.78 و 1.09 لتر/ساعة وحجم انتشار الدواء يساوي 14.67 ± 42.70 و 29 13.55 لتر بالترتيب.

بالنسبة ل 800 مليجرام حبوب للجرعة الأولى و الجرعة الاخيره كانت كالآتي: فترة نصف عمر الدواء تساوي 0.66 ± 4.00 و 2.15 ± 6.83 ساعة وأعلى تركيز في البلازما يساوي ± 17.21 7.35 و 7.53 ± 18.44 مايكروجرام/مل والزمن اللازم لملاحظة أعلى تركيز يساوي ± 2.17 0.41 و 0.82 ± 2.00 ساعة والمساحة تحت المنحنى تساوي 71.52 ± 121.56 و ± 264.56 136.96مايكروجرام.ساعة/مل ومتوسط فترة بقاء الدواء في الجسم يساوي 3.28 ± 7.86 و 12.49 5.29 ± ساعة ومعدل بقاء الدواء في الجسم يساوي 5.57 ± 8.79 و 2.48 ± 3.18 لتر/ساعة وحجم انتشار الدواء يساوي 27.11 ± 49.28 و 40.14 ± 36.66 لتر بالتبادل.

خلصت الدراسة إلى أن التمثيل الدوائي داخل الجسم للجرعات المختبرة يمكن مقارنته بصورة كبيرة مع التمثيل الدوائي داخل الجسم للمستحضرات المرجعية وهذه الدراسه تعزز من فكرة تراكم هذا الدواء فى الجسم عند الاستعمال المزمن وخاصة عند استعمال الجرعات الكبيره (< 400 ملجرام).

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Abbreviations

AUC_{0-16}	:	The area under the curve from zero to 16
AUC_{0-20}	:	The area under the curve from zero to 20
AUC_{0-24}	:	The area under the curve from zero to 24
$AUC_{0-\infty}$	:	The area under the curve from zero to infinity
CL	:	Clearance
C_{max}	:	The maximum drug concentration in the Plasma
C.V.	:	Confidence of variance
FDA	:	Food and Drug Administration in the United State of America
GIT	:	Gastrointestinal tract
HPLC	:	High performance Liquid Chromatography
Ke	:	Elimination rate constant
MRT	:	Mean residence time
PAR	:	Peak Area Ratio
Rt	:	Retention time
SD	:	Standard deviation
$T_{1/2}$	:	Half-life
T_{max}	:	The time required to observe C_{max} in the Plasma
Vd	:	Volume of distribution
µg	:	Microgram
SE	:	Standard error
h	:	hour
AUMC	:	area under the first moment curve
EEG	:	electroencephalogram
ng	:	Nanogram
nm	:	nanometer
r 2	:	regression
Cp_{ssmin}	:	minimum steady state concentrations
Cp_{ssmax}	:	maximum steady state concentration
λex	:	Excitation wavelength
λem	:	Emission wavelength
UV	:	Ultra Violet

AST (SGOT)	:	Aspartate aminotransferase (Serum glutamic-oxaloacetic transaminase)
ALT (SGPT)	:	Alanine aminotransferase (Serum glutamic-pyruvic transaminase)
GGT	:	Gamma Glutamyl Transferase
GGTP	:	gamma glutamyl transpeptidase

1. Introduction and literature review

Mycetoma is a major mycological health problem in tropical and subtropical areas.

Mycetoma is a chronic progressive subcutaneous granulomatous infection caused by tissue fungi or aerobic actinomyceas and hence it is divided into eumycetoma and actinomycetoma respectively (Mahgoub, 1989).

Mycetoma is characterized by tumefaction and formation of sinus tracts. The sinuses usually discharge purulent and seropurulent exudates containing grains. It may spread to involve the skin and the deep structures resulting in destruction, deformity and loss of function (Hay et al, 1992 and Mariat et al, 1977). French missionaries in Ponchicherry in India first recorded mycetoma in 1812; it was noticed there since 1714 (Corre, 1883). Gill (1842) of Madurai dispensary in Madras province, in Southern India, is frequently, mistakenly quoted as the first reporter of mycetoma in the medical literature. He described this condition as “a foot covered with large fungoid excrescence discharging an offensive ichorous fluid “(Gill, 1874).

In Sudan *Madurella mycetomatis* a true fungus, is the most common organism encountered (Fahal and Hassan, 1992).

1.1 The Etiology and Epidemiology of Mycetoma

Mycetoma is a chronic, relatively painless subcutaneous granuloma characterized by the formulation of multiple sinuses through which grains are discharged, the color of which depends on the etiological agents.

Mycetoma classified into two groups: Eumycetoma caused by tissue fungi and Actinomycetoma caused by aerobic actinomycetoma, which are higher filamentous bacteria. According to some workers a third group is classified Botryomycosis, which is caused by bacteria.

Various fungi, which produce grains of different sizes, colors and consistency, can cause Eumycetoma depending on the causative organisms. The most common ones include *Madurella mycetomatis*, *Madurella grisea*, *Leptosphaeria senegalensis*, *Pyrenochaeta romeroi*, and *Curvularia lunata*.

Actinomycetoma are mainly caused by aerobic actinomycetes which include *Streptomyces somaliensis*, *Actinomadura madurae*, *Actinomadura pellitieri*, and *Nocardia species*

The disease occurs in many parts of the world but more common in the tropical and subtropical countries of Africa, the Middle East and other parts of Asia and South America (Mariot et al, 1977).

Although India is considered as the birthplace of mycetoma yet the interest in mycetoma research had shifted at the turn of the century to the African subcontinent (Fahal, 1995). Gemy and Vincent form Algeria reported the first African case of mycetoma probably contracted the infection when he was in Tunisia. They isolated an aerobic actinomycete and streptothrix madurea (*Actinomadura madurae*) (Laveran, 1906). Pinoy in 1913 was the first to study actinomycotic agents and classified mycetoma organisms into two main groups the actinomycetes and the filamentous (true fungi) (Pinoy, 1913). Archibald in the Sudan in 1916 classified the etiological agents of mycetoma into two forms, eumycetes causing maduromycosis and pseudomycetes causing actinomycetoma (Chalmers and Archibald, 1916, Chalmers and Christopherson, 1916 and Chalmers and Archibald, 1918). Chiefly through the works of Mackinnon of Uruguay and the scientists of the Pasteur Institute in Paris, and Daker, the taxonomy of the causal fungi and actinomycetes had been considerably clarified (Mackinnon, 1954).

There is mycetoma belt, which lies between Latitudes 15° south and 30° north of the equator. Where there is a higher incidence of Savannah region than desert or tropical forests. In this region most of the rain falls during a season lasting 3-4 months. The remaining 8-9 are hot and dry which favor the growth of Acacia trees, which have big thorns that may play a role in introducing the organisms from soil into the body.

The belt includes Sudan, Somalia, Senegal, India, Yemen, Mexico, Venezuela, Columbia, Argentina and others (Mahgoub and Murray, 1973, Mariot, 1963 and Magana, 1984). In Africa, mycetoma is most frequently seen in Sudan, Senegal, Mauritania, Kenya, Niger, Nigeria, Ethiopia, Chad, Cameroon, Djibouti and Somalia (Abbot, 1956 and Lynch, 1964).

In Africa most mycetoma cases are eumycetoma caused mainly by *Madurella mycetomatis*. This is the most common agent of mycetoma in

Sudan, India and other tropical areas of Africa, but less in Somalia, Yemen and Saudi Arabia.

Actinomycetoma is more common than Eumycetoma in the Niger where the diseases prevails.

In the Northern deserts zone, the disease is predominantly caused by *Streptomyces somaliensis*, where as in the southern part, it is caused by *Actinomadura pellitieri*, followed by *Nocardia brasiliensis* and *Actinomadura madurae* in descending order of frequency (Develoux et al, 1985).

Streptomyces somaliensis is seen more often in the Middle East, Central and West Africa and the arid region adjacent to the Sahara desert. *Actinomadura pellitieri* is more prevalent in the relatively humid areas. Nocardia are usually the causative organisms of mycetoma in temperate regions. Few cases have been reported in Upper Egypt and where all caused by *M. mycetomatis* (El-Mofti et al, 1965).

In central Europe, the disease is rare but cases have been reported from Italy and Romania.

The most common case of eumycetoma in the USA is *Petriellidium boydii*, which have been isolated from soil in the USA and Canada.

1.2. Mycetoma in Sudan

Mycetoma seems to have been known in Sudan before the advent of modern medicines, by its present common name of “Nebit” meaning growth. “Fakkis” practiced treatment by cauterization and/or amputation during the time of the Mahdeya (1885- 1899) (Mahgoub, 1994).

Balfouer published the first documented report of a case of mycetoma in Sudan in 1904 (Balfouer, 1904)

In 1931, Grantham–Hill, the senior surgeon at Khartoum Hospital made a detailed clinical study of 184 cases out of which 64% were of the black variety and 36% were yellow. Noting that the yellow type is actinomycotic and the black type is maduromycotic, he discussed the relative virulence of the two types. He doubted the value of medical treatment by various drugs suggested up to that date. He thought that the best routine treatment was

surgical, and that the key to success lies in early recognition and complete removal.

Grantham–Hill (1931) was aware that amputations will deter patients from attending to hospital and therefore encouraged early detection. He therefore, defended local removal as much as possible. In his series of 184 cases, 141 (77%) were treated by local removal and 43 (23%) by amputation. He gave support to the common belief that “Nabit” usually follows a thorn prick by saying that in 30% of his patients with mycetoma of less than six months duration; thorns were actually found embedded in the growth after removal at operation.

Dr. Albert Chalmers, Director of Wellcome Tropical Research Laboratories, later known as Stack Laboratories and then the present day National Health Laboratories Khartoum, to gather with Captain R.G Archibald, pathologist, and Dr. J.B. Christopherson, Director of Khartoum and Omdurman Civil Hospital, carried out extensive studies of two causal organisms. Chalmers and Archibald gave quite an elaborative and specific definition of mycetoma as “All growth and granulations producing enlargement, deformity and destruction in any part of man, brought about by the invasion of the affected area by certain species of fungi belonging to different genera which gave rise to variously colored and shaped bodies called “ grains” which were formed of hyphae with or without chlamydospore, and are found either embedded in the pathological tissue forming the growth and granulation or escaping in the in the discharge from the diseased area” (Chalmers and Archibald,1916).

For the first time Chalmers and Archibald introduced the terms Maduromycosis and Actinomycoses, preceded afterwards to classify the mycetoma into Maduromycetoma the grains of which are composed of large segmented mycelia and Actinomycetoma with grains composed of fine non-segmented filaments (Chalmers and Archibald, 1918).

Abbott carried out in vitro trials with antibiotics against *Madurella mycetomatis* and *Streptomyces somaliensis*. He found that the growth of *M. mycetomatis* was unaffected by chloramphenicol, oxytetracycline, carbomycin and polymyxin B. While *S. somaliensis* was markedly sensitive to these antibiotic except Polymyxin B (Abbot, 1956 and Mahgoub, 1994).

Abbott noted that atrophic and neurological changes were absent in mycetoma and on dissection of some of the mycetomatis; glistening white tendons and nerves lay unaffected in the middle of damaged tissue.

In 1964 Mahgoub published for the first time an article on the serological diagnosis of mycetoma. He also obtained a Ph.D. in 1965 on Mycotic infections in Sudan, the major part of which was mycological and serological study of mycetoma (Mahgoub, 1965).

The late Mr. Ibrahim Moghraby (1967) who worked for many years as a surgeon in Wad Madani Hospital, center of endemic area for mycetoma, presented to the 6th Arab Medical Conference in Khartoum a study on "Mycetoma in the Gazira, a Public Health Problem" (Mohgraby, 1967).

Heinemann Medical books of London in 1973 published a monograph on mycetoma written by Mahgoub and Murray.

In Sudan, *Madurella mycetomatis* accounts for 71.4% of all mycetoma cases, *Streptomyces somaliensis* for 18%, *Actinomadura madurae* 5.3% and *Actinomadura pellitieri* for 2.7% (personal observations, Gumaa. SA) (Figure 1-1).

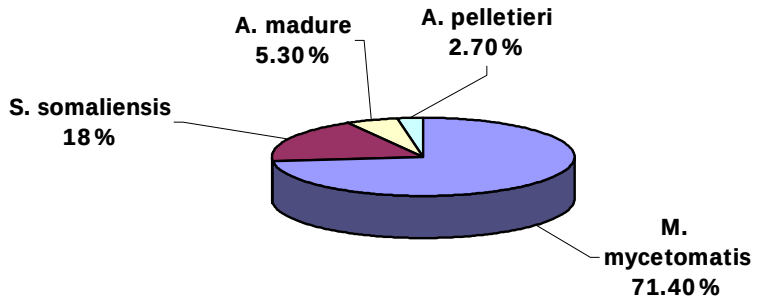

Figure 1.1. Distribution of mycetoma agents in Sudan

Since mycetoma agents have been isolated from soil and vegetation in endemic areas, naturally the diseases prevails in those people who come in direct contact with the soil like farmers, cultivators, wood cutters, and herdsmen, but no occupation is exempted.

In Sudan, males are affected more than females in the ration of 5:1 (Abbott, 1956) (Figure 1-2).

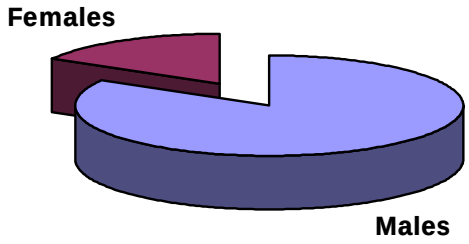

Figure 1.2. Gender distribution of mycetoma in Sudan

This is genuine gender difference and is not related to the great or outdoor activities of males because in certain areas of Sudan, males and females go out to work in the fields side by side. The disease is more common between the age group 20-40 years; this being the earning group, but no age is immune.

The problem of treating mycetoma due to *Madurella mycetomatis* remained unsolved for quite a time, and once more success of treatment with Ketoconazole was reported form Sudan and Saudi Arabia by Mahgoub and Gumaa (1984) (Mahgoub and Gumaa, 1984) in a series of thirteen patients five were cured and four were greatly improved by daily intake of Ketoconazole.

Since then, many environmental, social and economical changes have taken place. Global variation in the pattern of mycetoma and changes in the disease spectrum over the years are well documented in the literature. Never the less, there is a need for periodic studies of mycetoma to define these changes so as to improve the management of mycetoma patients.

Mycetoma lesions are amenable to surgical excision but the recurrence rate is high. Advanced cases usually require prolonged medical treatment. However, in spite of the use of conventional therapeutic doses for a long duration, the response to medical treatment in many patients especially those with eumycetoma is delayed and far from satisfactory (Mahgoub, 1976 and Welsh et al, 1987). Possible factors that influence the effectiveness of chemotherapy in mycetoma include the sensitivity of the organism to the drug, drug Pharmacokinetics and the local blood supply to the lesion.

Actinomycetoma due to *Actinomadura pellitieri* is an uncommon infection, accounting for less than 3% of all mycetoma cases in the Sudan (Fahal and Hassan, 1992).

The subject of Mycetoma since then became the focus of study for some Sudanese researchers where intensive investigations had been carried out (Fahal, 1994).

1.3. Route of entry

The organisms are usually present in the soil in the form of grains. After they are moistened by rain, they form conidia or other forms able to infect the host. This infecting agent is then implanted into the host tissue through a breach in skin produced by trauma caused by sharp object such as thorn pricks, stone or splinters. However, in many patients there is no history of trauma at the site of infection. In areas where mycetoma is frequent the habit of going barefooted is common and thorns are plentiful. As a result, natural infection is expected to be more frequent than it actually is, if this theory of route of infection is true. The disease is not contagious from person to other or from animal to human (Mahgoub and Murray, 1973, Abbott, 1956 and Lynch, 1964).

1.4. Incubation period

The rate of progress is more rapid with actinomycetoma than with eumycetoma. In eumycetoma, the lesion grows slowly with clear defined margins and remains encapsulated for a long period, whereas, in actinomycetoma the lesion is more inflammatory, more destructive and invades the bone at an earlier period (Gonzalez, 1975, Hay et al, 1992, El Moghraby, 1971 and Grantham- Hill, 1968). The incubation period in mycetoma is unknown due to the difficulty in establishing the time of initial infection however in experimental animals the formation of the granuloma was noted after a period of three weeks from the inoculation of the organism (Mahgoub and Murray, 1973, Magana, 1984 and Lynch, 1964).

1.5. Mycetoma immunology

Antibodies against mycetoma organisms usually develop after the infection. They are of two types, the precipitating and complement fixed ones. The antibodies rise with the disease activity, decline with recovery and disappear with cure. They can be used for the diagnosis of mycetoma, identification of the individual organism and follow up of patients on medical treatment (Gordon and Hagan, 1936).

1.6. The Pathology of Mycetoma

Mycetoma (maduromycosis) is a chronic granulomatous subcutaneous infection caused by actinomycete (actinomycetoma) or by true fungi (eumycetoma). It is characterized by the formation of aggregates of the organism (grains) in the tissues, which are visible to the naked eye. The grains vary in color, size and consistency depending on the mycetoma causative agent (Figures 1-3 to 1-6).

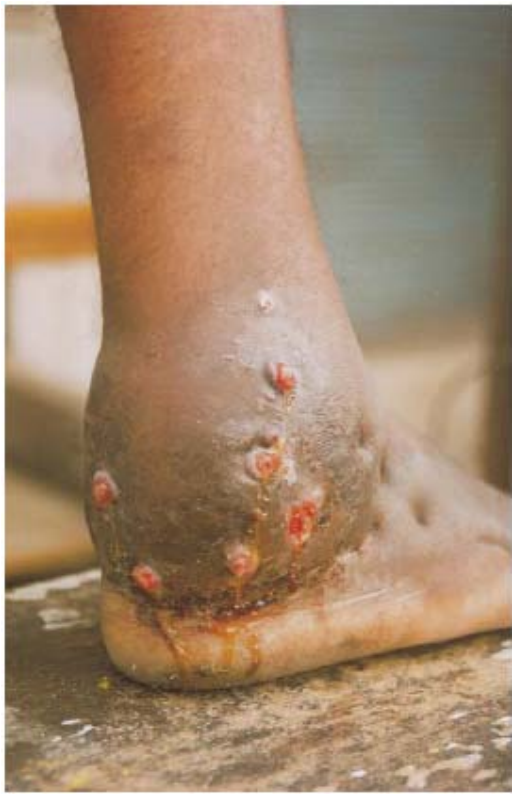

Figure 1.3. Mycetoma of the foot

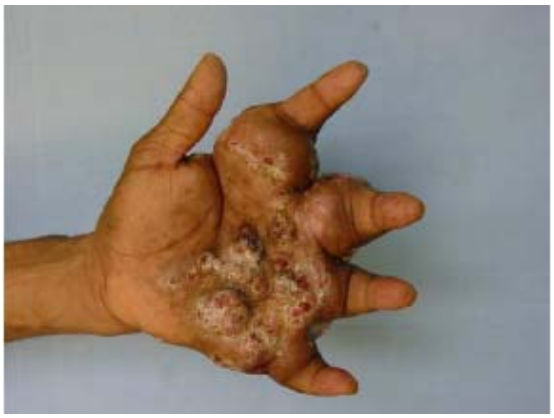

Figure 1.4. Mycetoma of the hand

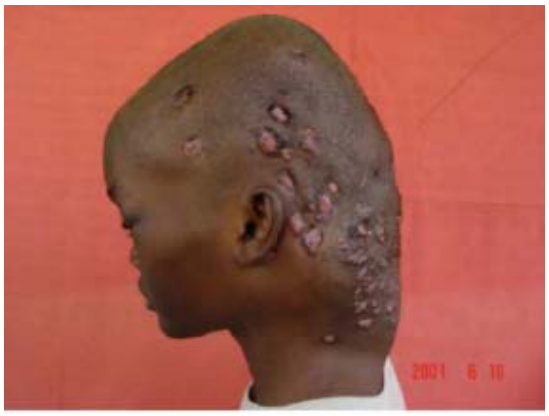

Figure 1.5. Mycetoma of the head

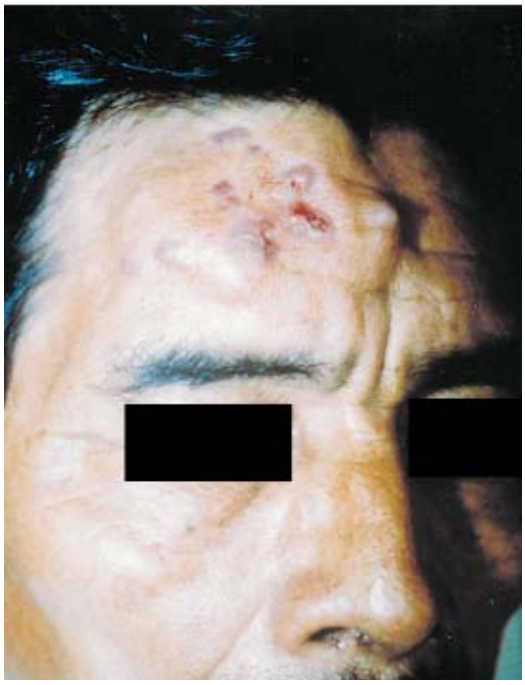

Figure 1.6. Mycetoma of the face

Both actinomycetoma and eumycetoma prevail in the Sudan. Eumycetoma due to *Madurella mycetomatis* is the most common type (Mahgoub and Murray, 1973 and Lynch, 1964).

The actinomycetoma is commonly caused by *Streptomyces somaliensis*, *Actinomadura madurae* and *Actinomadura pellitieri*.

1.7. Clinical Presentation of Mycetoma

Mycetoma is seen in males more frequently than in females with a ratio of 5:1. This is a genuine difference and it is not related to the outdoor activities as was previously considered, due to the fact that in endemic areas, females are committed in outdoor activities as much as males (Mahgoub and Murray, 1973 and Cameron et al, 1973). No age is exempted but mycetoma commonly affects adults between 20-40 years of age, the earning members of the society.

Mycetoma is seen more conventionally in cultivators, field laborers and in herdsmen who come in contact with the land as seen in Africa nomads and the Arabian Peninsula (Mahgoub and Murray, 1973 and Abbot 1956).

The duration of the disease at presentation varies between three months to 30 years, patients tend to present late with advanced disease. This may be due to the nature of the disease, which is painless and slowly spreading and lack of health education.

The rate of progress is more rapid with actinomycetoma than with eumycetoma. In eumycetoma, the lesion grows slowly with clearly defined margins and remains encapsulated for a long period whereas in actinomycetoma the lesion is more inflammatory, more destructive and invades the bone at an earlier period (Mahgoub and Murray, 1973, Chadfeild, 1964 and Manson 1987). This is clearly seen in infection with *Actinomadura pellitieri* which is more cellular, infiltrative destructive and it is a potent osteophytic agent (Culligan et.al, 1985 and Kamalam and Thambiah, 1987). This is also observed with *Nocardia brasiliensis* (Hogshead and Stein, 1970). One or a combination of the following factors may influence the clinical picture of mycetoma, duration of the disease, causative organism, site of infection and immune response of the host.

The characteristic triad of a subcutaneous nodule, sinus and the presence of grains are pathogonomic of mycetoma. It presents as a slowly progressive painless subcutaneous swelling at the site of previous trauma (Mahgoub and Murray, 1973, El Moghraby 1971 and Grantham–Hill, 1934). The swelling is usually firm and rounded but it may be soft, lobulated, and rarely cystic and it is often mobile.

Mycetoma is usually painless in nature because it is assumed that the organism produces substances, which have an anesthetic action (Gumaa, 1983). In about 18% of cases, patients may seek medical advice for the pain, which may be produced by the expansion of the bone with the mycetoma or due to secondary bacterial infection (Fahal and Hassan, 1992).

For unknown reasons, the tendons and the nerves are curiously spared until very late in the disease process, this may explain the rarity of neurological and atrophic changes in mycetoma (Gumaa, 1974).

The most common site for mycetoma is the foot (Figure 1-3) which accounts for 70% of cases, most of the lesions are seen on the dorsal aspect of the forefoot and for unexplainable reasons the left foot is more affected (Mahgoub and Murray, 1973, Magana, 1984, Abbot, 1956 and Fahal and Hassan, 1992). The hand (Figure 1.4) is the next common site, which occurs in 12% of cases (Murray IG, 1960 and Tight and Bartlell, 1981). In endemic areas other parts of the body may be involved but less frequently, the knee, arm, leg, head and neck (Figure 1.5), thigh and the perineum are affected in that order (Soretain et al, 194 and Brijesh et al, 1984). Rare sites such as the chest, abdominal wall, facial bones (Figure 1.6), mandible, testes, paranasal sinuses, eyelid and surgical incisions may be affected (Aldrige and Kirk, 1940 and Fahal et al, 1994).

Figure 1.7. Most common sites for mycetoma

Mycetoma in general involves those parts of the body that come in contact with soil during standing, sitting or lying down. Visceral mycetoma had not been reported yet.

1.8. Medical Treatment of Mycetoma

The morbidity of mycetoma in Sudan has not changed and perhaps more than 400 new cases are seen in hospitals and outpatient clinics every

year (Sudan medical journal, 1994). Similarly the outcome of surgical treatment alone has remained as leading to 80% recurrence rate. The quest for effective medical treatment was therefore actively perused. In vitro tests with antibiotics were carried out on mycetoma organisms for the first time in Sudan in 1956 (Abbot, 1956). At the same time there were reports of successful medical treatment with chemotherapeutic agents of mycetoma caused by *Nocardia brasiliensis* from Mexico (Gonzalez–Ochoa, 1955). Against this background the mycetoma Clinic at Khartoum North Civil Hospital was established in 1968, in order to conduct clinical trials of current and the would be available drugs.

Prior to starting medical treatment it is absolutely necessary to determine whether the causal organism of the mycetoma is a bacterium (actinomycetoma) or a fungus (eumycetoma). Many failures are attributed to giving the treatment of one to the other.

It is as well worth noting that surgical reduction of a bulky swelling without affecting the function of an affected bony lesion will certainly reduce the length of treatment.

1.8.1. Treatment of Eumycetoma

In many centers, surgery is the most acceptable line of treatment for eumycetoma cases: in the form of aggressive surgical excision or amputation in advanced disease (Mahgoub and Murray, 1973, Magana, 1984 and Lynch, 1964).

Reports of medical treatment in eumycetoma are few (Murray and Colichon, 1962). Various antifungal agents have been tried with little success. This is perhaps surprising, as the fungi causing eumycetoma are low-grade pathogens and their eradication should be readily achieved by the administration of safe systemically active agent (Murray and Colichon, 1962). Amphotericin B has been used with limited success and it is no longer popular due to its toxic side effects (Mahgoub and Murray, 1973). In the case of eumycetoma due to *Madurella mycetomatis*, which constitutes the major causative agent in tropical and subtropical Africa and Asia, the drug of choice is ketoconazole (Mahgoub and Gumaa, 1984).

A number of studies showed that ketoconazole could cure eumycetoma patients. Mahgoub and Gumaa reported, in 1984, impressive results in treating thirteen patients with confirmed eumycetoma. Five of them were cured, two of these patients had bone involvement, four patients showed considerable improvement with ketoconazole. The dose for the cured patients was 300- 400 mg daily, while the dose for those who improved was only 200 mg daily.

Treatment of these patients continued for periods ranging from three months to three years (Mahgoub and Gumaa, 1984).

In a dose of 200 mg twice daily, no significant side effects or biochemical abnormalities are seen. Ketoconazole is, however, not effective in mycetoma caused by *Petriellidium boydii* or *Acremonium species* (Hay et al, 1992). Liver function tests are regularly carried out every month during treatment.

Other regimes include procaine penicillin in a dose of 600000-800000 units per day given intramuscularly and griseofulvin in a dose of 500 mg three times a day given orally.

1.8.2. Treatment of Actinomycetoma

Actinomycetoma is amenable to medical treatment with antibiotics and other chemotherapeutic agents. Combined drug therapy is always preferred to a single drug (Hay et al, 1992, Mahgoub, 1976 and Mahgoub and Murray, 1973). Standard treatment is a combination of streptomycin and cotrimoxazole, but if there is no response, a combination of streptomycin with one of the following drugs is given: dapsone, sulphadoxine-pyrimethamine (Fansidar®) and rifampicin. It is reasonable to start with a combination of streptomycin sulphate in a dose of 14 mg/kg daily for one month then on alternate days and diaminodiphenyl sulphone (dapsone) in a dose of 1.5 mg/kg twice daily for patients with *Streptomyces somaliensis*, *Actinomadura madurae*, and *Nocardia species*. If there is no response for few months or if there was persistent side effect then dapsone is replaced by Co- trimoxazole (Septrin®) in dose of 1.5 mg/kg twice daily (Mahgoub, 1972 and, 1976).

Actinomadura pellitieri usually resistant to the dapsone and the combination of choice is streptomycin sulphate and Septrin (Mahgoub, 1976). Whatever regimen is used, supportive treatment with ferrous sulphate and folic acid and regular follow up of patients for hemoglobin concentration and total white blood count must be provided.

Treatment must be continued until the patient is clinically, serologically and radiologically cured. Clinical improvement is judged by reduction in the size of the mass and healing of the sinuses. Cure is considered when the skin becomes normal, the mass disappeared, the sinuses had healed and the organism is eliminated form the tissue. Photography of the lesion is usually offered every 3 months or less for follows up.

Medical treatment should be given pre and post operatively as it facilitates surgery, accelerates healing and reduces the chance of relapse. The cure rate varies between 60-90% (Fahal and Hassan, 1992, Hay et al, 1992, Mahgoub, 1976 and Mahgoub and Murray, 1973).

Recurrence is more common after an incomplete course of medical treatment and there is a good chance for the organism to develop drug resistance.

Actinomadura pellitieri responds better to combination of streptomycin and cotrimoxazole, whereas *Actinomadura madurae* responds better to streptomycin and dapsone. *Nocardia asteroides*, *Nocardia brasiliensis* and *Streptomyces somaliensis* respond to either drug combination.

The drug of choice in the treatment of eumycetoma is ketoconazole. The length of treatment varies form 6-12 months depending on size of mycetoma and response to treatment.

Mycoserology has been developed using techniques such as counter-immuno–electrophoresis (CIE), enzyme linked immunosorbent assay (ELISA) and immunodiffusion. These approaches are important.

1.9. Surgical Treatment of Mycetoma

In the Sudan *Madurella mycetomatis*, which is a true fungus, is the most common organism encountered. *Streptomyces somaliensis*, *Actinomadura madurae* and *Actinomadura pellitieri* are the most common

causative organisms for actinomycetoma (Fahal and Hassan, 1992). Mycetoma is endemic in the central part of the country and it is rarely seen in the South (Abbott, 1956 and Lynch, 1964).

The disease presents as a localized tumor – like mass with or without sinuses. Mycetoma progresses slow eventually it leads to the destruction of the deep tissue and bone. The most common site is the dorsum of the foot hence the name “Madura foot”. However extra- pedal involvement is seen as well, it was reported in the hand, leg, head and neck, abdominal wall, buttock and perineum (El Moghraby, 1971 and Gumaa, 1983).

Surgery for mycetoma ranges from local excision to mass reduction and occasionally amputation of the affected part.

The aim of surgical treatment is complete removal of the lesion, this is possible in patients who have early disease in which the granuloma is localized and confined to the subcutaneous tissue (Mahgoub and Murray, 1973). In patients with massive lesion, mass reduction is indicated, in these cases the involved soft tissue is excised and the bone is curettage. The technique greatly facilities the response to medical treatment.

To avoid the socially disastrous consequences of amputation, a program of extensive repeated excision of the diseased tissue, including bone may be carried out preferentially (Bandl et al, 1987). This debunking procedure must be accompanied by mycetoma chemotherapy.

The recurrence rate with surgery is high, ranging from 20 to 90 percent (Fahal and Hassan, 1992 and El Moghraby, 1971). Medical treatment before surgery is recommended to ascertain high level of the drugs in the circulation to minimize the chance of the organism local spread. In most cases medical treatment should continue until the patient shows clinical, radiological and serological evidence of cure.

Eumycetoma is well encapsulated and great care must be exerted not to rupture the capsule to minimize the spread of grains along the fascial planes if recurrence is to be avoided.

Actinomycetoma has an ill-defined border therefore a margin of health tissue should always be excised with the lesion.

1.10. Assessment of Progress and Follow up of Mycetoma Patients

Both Subjective and objective criteria must be taken into consideration for the assessment of response to treatment. In the former, the patients's own assessment of his progress as well as that of the nursing sister and the treating doctors is recorded.

For the objectives criteria, patients are questioned about pain before and during treatment, and the number and activity of sinuses is recorded as well as the appearance of the skin. On examination, the skin is usually attached to the underlying tissue. When the patient starts to show clinical improvement the overlying skin becomes less fixed and the underlying mass becomes freely mobile.

The circumference of the swelling is measured by means of a tape measure and whenever possible this is done at fixed bony prominences like the malleoli or at a measured distance from the tip of the toes. More permanent records are kept by taking photographs of the same view at a fixed distance before, at different intervals during, and at end of treatment. At about three months intervals radiographs of the affected lesion are taken to assess bone improvement.

Sera are collected at monthly intervals and tested by means of counter-immuno-electrophoresis (CIE). After staining with naphthalene black, both the number and intensity of the precipitation lines are recorded. A patient is considered cured when his serum becomes negatives and remains so in two successive precipitation tests (Mahgoub, 1985).

1.11. Recurrence in Mycetoma

Recurrence is either local at the site of previous lesion or at the regional lymph nodes. The postoperative recurrences rate in mycetoma various from 25 to 50% (Hay et al, 1992, Mahgoub and Murray, 1973 and Lynch, 1964). It is mainly due to inadequate removal of the organisms during surgery.

Postoperative medical treatment is essential because if the treatment is stopped after apparent cure, recurrence may be immediate. Follow up of

patients with mycetoma must be long enough to detect early recurrence and to advise early treatment (Mahgoub, 1976).

1.12. Mycetoma Research in Sudan

The Sudan seems to be the homeland of mycetoma. Lynch in 1964 estimated the incidence of mycetoma in the Sudan as 300 – 400 new hospital cases annually. This figure does not take into account the considerable number of outpatients and those seen at peripheral dispensaries where qualified doctors might not even be present.

A mycetoma clinic at Khartoum North Civil Hospital was established in 1968 in order to conduct clinical trials of current and potential drugs.

Investigation, follow up and documentation of mycetoma cases in Sudan were made feasible by the establishment of the Mycetoma Research Center at Soba University Hospital (Fahal, 1994). The center provides diagnosis, medical treatment and follow up of mycetoma patients from almost all over Sudan.

1.13. Antifungal Agents

1. 13.1. Introduction

Fungi live all around, they mainly exist in soil, converting lignin, chitin, keratin, and cellulose into humus, but they also grow on plants, thrive in the fur of pets, and help the body maintain normal functionality. These heterotrophic microorganisms can, however, cause serious illnesses, termed mycoses, in humans. Mycotic illnesses in humans are divided into two basic categories: systemic mycoses and superficial mycoses. The former are non-contagious and can be quite serious. Examples include aspergillosis and blastomycosis. Superficial mycoses can be further divided into the categories of dermatophytic infections and candidosis. Dermatophytic infections affect the skin, hair, and nails. The fungi digest the keratin in the tissues just as they do in the soil. Scalp ringworm and athlete's foot are examples of this type of contagious infection. Candidosis affect moist skin and mucous membranes.

Candida albicans naturally exists in our gastrointestinal tract and aids in digestion, however this fungus can spread to other parts of the body, causing infection. An example is vaginal candidosis, which is only contagious to a fetus, should the infected woman be pregnant.

The drugs used to treat mycoses are either applied topically at the site of infection, or delivered systemically. The major families of antifungal agents include antibiotics, antimetabolites, the azoles and synthetic allylamines. The key event of antifungal drug development is shown in figure 1-8.

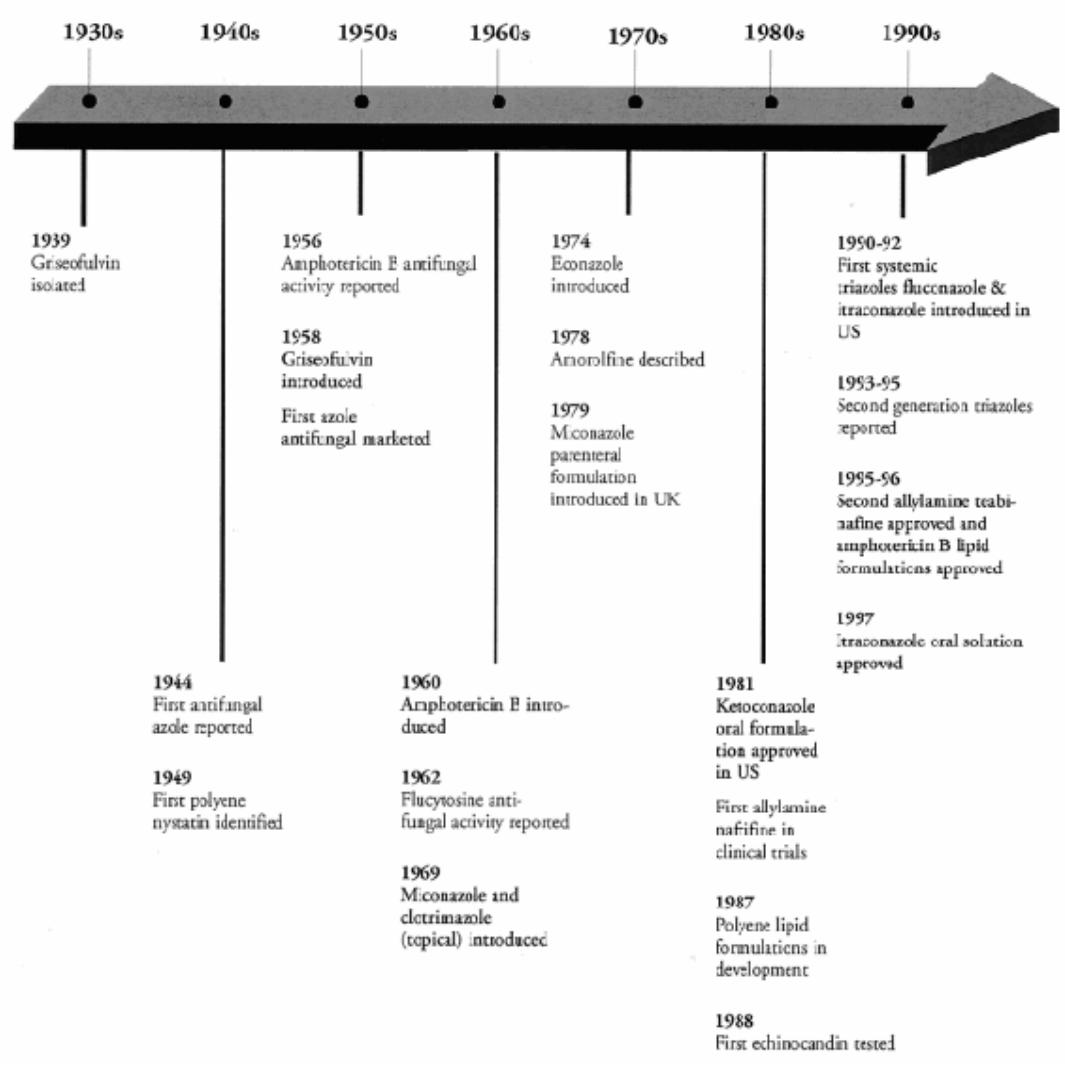

Figure1.8. Key events in antifungal drug development.

1.13.2. Antibiotics

Various types of antibiotics have proven useful in controlling fungal infections. Griseofulvin was first obtained from *Penicillium griseofulvum* in 1939 and has been used systemically to treat dermatophytes (Figure 1-9). Since it is administered orally, but because this drug is rather insoluble in water, absorption is very low. Taking the tablets with a high-fat meal is recommended as it increases absorption.

It's fungistatic, rather than fungicidal, activity is limited to actively growing dermatophytes, and it is most effective in the upper body where continuous reinfection is least common. Griseofulvin has a dual effect on fungal cells. First, it binds to the RNA, preventing replication. Secondly, it causes multipolar mitosis, producing abnormal nuclei and thus abnormal fungi. As a result of this action, hyphal penetration is substantially inhibited, allowing the outward thrust of keratinized host cells to deprive the fungi of the required nutrients.

Side effects in humans include headache, allergic, neurological, and gastrointestinal reactions, and 1ncreases in feca1 protoporphyrin. It also induces hepatic microsomal enzymes, which retard the activity of oral anticoagulants. In addition, teratogenic and carcinogenic activity has been observed in lab animals. As a result, Griseofulvin is only used as a last resort for treating superficial dermatophyte infections.

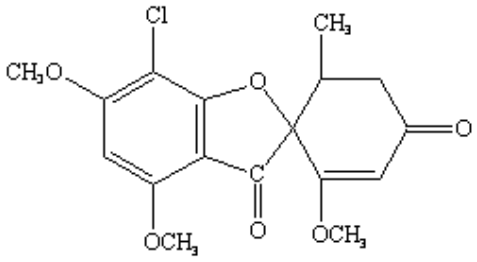

Figure1.9. Chemical structure of griseofulvin

A class of antibiotics known as the polyenes is useful in the treatment of candidacies and systemic mycoses. Polyenes contain a large lactone ring

with four to seven unsubstituted, conjugated double bonds. These are usually in an all-trans configuration, creating a planar molecule. These compounds are rather insoluble in water, and, due to the vast amount of double bonds, somewhat reactive. amphotericin B and nystatin (Figures 1-10 and 1-11, respectively) are prime examples. Amphotericin B prevailed as the dominant treatment for systemic mycoses for more than two decades after its development in the 1950's. Due to its low absorption in the gastrointestinal tract, intravenous administration is necessary. Nystatin is toxic in the human body, thus it can only be applied topically. It has been very effective in treating superficial mycoses such as vaginal candidosis.

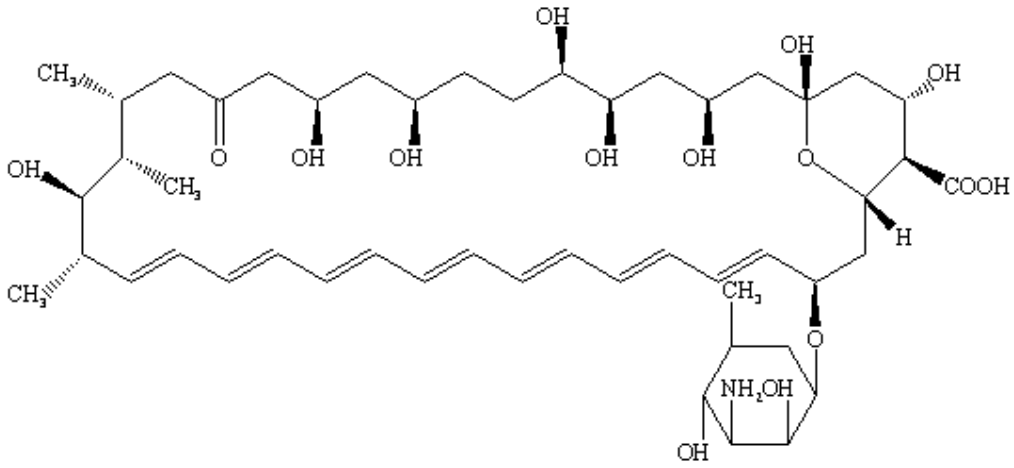

Figure1.10. Chemical structure of amphotericin B

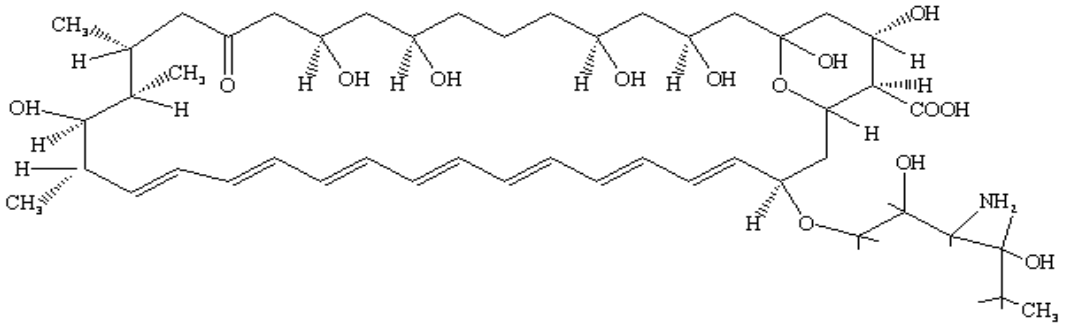

Figure1.11. Chemical structure of nystatin

These antibiotics, being lipophilic, act by binding to sterols in the membranes of cells. This includes cholesterol in animal cells and ergosterol in fungal cells. When the molecules bind to the cell membrane, its permeability is altered. As a result, the cell can no longer control its internal environment-ions and organic cell constituents can enter and leave uncontrolled. Fortunately, these compounds have a greater affinity for ergosterol than for cholesterol; however, severe side effects can occur: nausea, anoxeria, chills, fever, headache, anemia, phlebitis, hypokalemia, and azotemia. The greatest drawback, though, is its toxicity in the kidneys. When administered, the dosage must be carefully monitored so that no more is in the blood than is necessary. Lower dosages have also been given with 5-Fluorocytosine with positive effects (Figure 1-12).

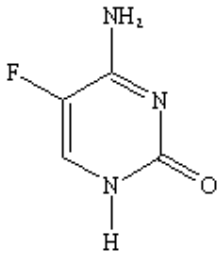

Figure1.12. Chemical structure of 5-flucytosine

1.13.3. Antimetabolites

Flucytosine (Figure 1-12) is a prodrug, which is highly effective, but only against a narrow spectrum of fungi, namely yeasts. It is applied systemically, where it is converted to 5-fluorouracil by a specific deaminase found only in yeast cells. As a result, this drug is highly selective. In addition to its high selectivity; this drug takes two courses of action against the infecting fungi. First, the 5-fluorouracil is incorporated into the RNA of the fungi as though it were uracil. This leads to severe genetic mutation. Secondly, the 5-fluorouracil is metabolized to 5-fluoro-2'-deoxyuridylate, which inhibits thymidylate synthetase, an enzyme necessary for the production of thymidine. Thymidine is required for DNA synthesis. These two mechanisms

make it a very effective agent for systemic treatments of Candida, Cryptococcus, and Torulopsis infections.

This drug is typically taken orally, where it achieves rapid and complete absorption. It distributes throughout the body, including the cerebrospinal fluids, which explains its effectiveness against meningitis infections. Flucytosine is often given in conjunction with Amphotericin B. It is believed that the polyene damages the fungal cell wall sufficiently to allow more 5-FU in, expediting its desired effect. A major drawback of 5-fluorouracil is the rapid development of resistant strains - yet another reason to combine it with another treatment.

As 5-fluorouracil is also an anticancer drug, the side effects can be rather strong. It can cause bone marrow depression (including leukopenia and anemia), gastrointestinal disturbances (such as nausea, vomiting, and diarrhea), and central nerves system toxicity (which can produce headache, drowsiness, and hallucinations).

1.13. 4. Azoles

The azole family consists of compounds containing one or more imidazole or triazole ring.

The azoles quickly gained popularity, as they presented systemic and topical treatments effective against nearly all mycoses. Clotrimazole and miconazole (Figure 1-13), both developed in 1969, were the first imidazoles used in antifungal chemotherapy.

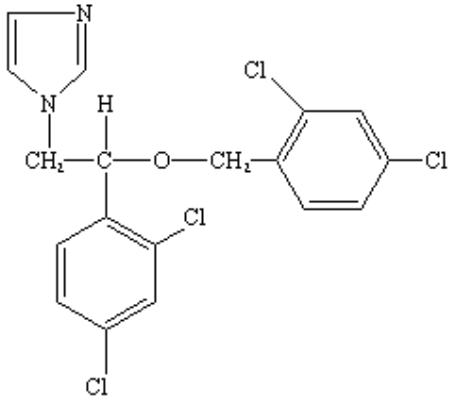

Figure1.13. Chemical structure of miconazole

The azoles target a fundamental difference between mammalian and fungal cells-the production of the sterol used in the cell membrane. Sterols are of particular importance to cells, as they control the structure and permeability of the membrane. While mammalian cells use cholesterol, fungal cells have ergosterol. There is a common biosynthetic pathway shared by mammals and fungi up a certain point, and it is a specific step in the synthesis which the azoles block.

Both mammals and fungi produce lanosterol and use a 14 α-demethylase to begin converting it into their respective sterol. Fortunately, this enzyme is slightly varied between mammalian and fungal cells, allowing a modified azole the ability to select for fungal cells. Because the 14 α-demethylase system is carbon dioxide sensitive, it requires cytochrome P-450 to initiate the oxidation of the 14 α-methyl of lanosterol. At the core of cytochrome P-450 lays an iron molecule bound to four N molecules. It is to this iron molecule that the azole binds, destroying its oxidative potential. This thereby prevents the demethylation of lanosterol and thus ergosterol cannot be produced. Such inhibition leads to the breakdown of the fungal cell membrane. This mode of action is confirmed by the observation of an increased concentration of 14 α-methylsterols within treated fungal cells.

Clotrimazole and miconazole both showed great activity as topical creams, but serious side effects (such as anemia and thrombocytosis) arose from systemic administration. In addition, much of the drug was lost through first-pass metabolism in the liver, and, due to its lipophilic nature, the drug would bind to proteins in the blood so that little was available to have its antifungal effect. The azoles, however, showed much promise, and other drugs were developed which were tolerated by our system and effective against infections.

Ketoconazole was the next imidazole to become widely used, as it had the most widespread effect and the fewer side effects. This drug is available as a prescription table, a cream, or a shampoo, and represents a major step in systemic treatment of mycoses. Unlike its predecessors, ketoconazole is readily absorbed and delivered throughout the body. Some common side effects include headache and nausea, but more severe are its effects on

cholesterol and testosterone levels. Ketoconazole, in small doses, has been shown to lower cholesterol in humans, presumably because its specificity factors are not sufficient to distinguish between the mammalian and fungal enzyme systems. This drug has also been shown to inhibit adrenal corticosteroid and testosterone synthesis. As it has these effects, ketoconazole has actually been used to treat adrenal hyperplasia and cancer. All systems return to normal once treatment is stopped.

Itraconazole and fluconazole (Figures 1-14 and 1-15, respectively) are two triazoles that represent the next major developments.

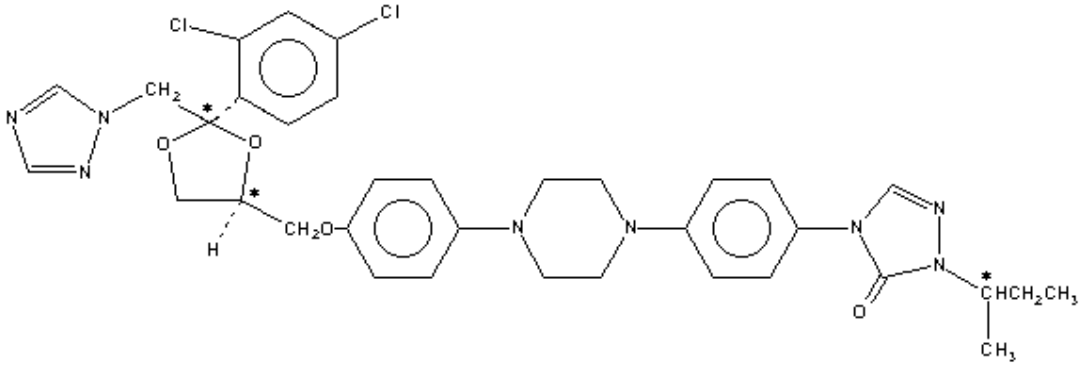

Figure1.14. Chemical structure of itraconazole

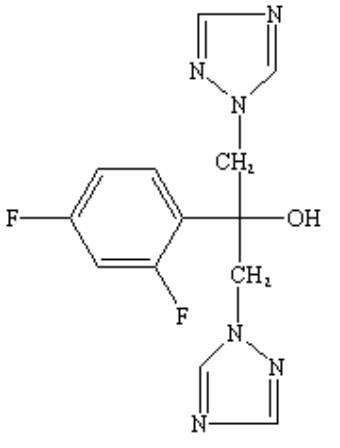

Figure1.15. Chemical structure of fluconazole

Itraconazole showed greatest activity against dermatophytic infections of the skin and nails, while fluconazole showed promise with yeast infections such as candidosis and Cryptococcal meningitis. Fluconazole is particularly noted for its lack of serious side effects and its compatibility with azithromycin (before fluconazole, AIDS patients had few if any options for dealing with fungal infections). One reason for this could be its relative stability in the system-the C-F bonds of the benzyl ring are quite strong and rather unreactive. Fluconazole is also a highly active antifungal agent-it is absorbed 100% into the body and is 10-100 times more effective than ketoconazole.

1.13.5. Synthetic Allylamines

This family of antifungal agents was discovered in 1979 and presents many valuable advances. Terbinafine (Figure 1-16) is an example of an orally administered allylamine, and naftifine and pyridone ciclopirox are examples of a topical cream and gel.

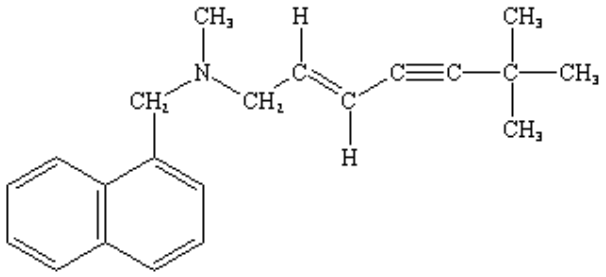

Figure1.16. Chemical structure of terbinafine

The systemic drugs act by inhibiting squalene epoxidase, which converts squalene into squalene oxide. This oxidation represents the first step in the synthesis of ergosterol, which is a required constituent of the fungal cell membrane. In addition, squalene is toxic to the cell, and if it is not converted, the levels build up within the cell and rapidly kill it. The topical drugs act by accumulating within the cell and interfering with amino acid transport through

the cell membrane. This instability leads to cell death. These molecules also have the ability to penetrate into hair follicles and prevent reinfection.

The allylamines are most effective against filamentous fungi, such as found in dermatophytic infections like Tinea corporis and Tinea unguum. Some of the great advantages of this drug over previous treatments include shorter term of treatment, fewer and milder side effects, fewer drug interactions, and greater potency.

A Schematic description of sites of action of different antifungal agents is shown in figure 1-17. Candins cause disruption of cell wall, allowing other antifungals (polyenes, azoles, and 5-FC) to enter. Azoles and polyenes can inhibit or bind to ergosterol, leading to cell lysis and allowing 5FC to enter the cell and inhibit nucleic acid synthesis. Dashed arrows indicate the site of action for each antifungal class. The mechanisms of action for each class of antifungal agent are depicted in panels A through D.

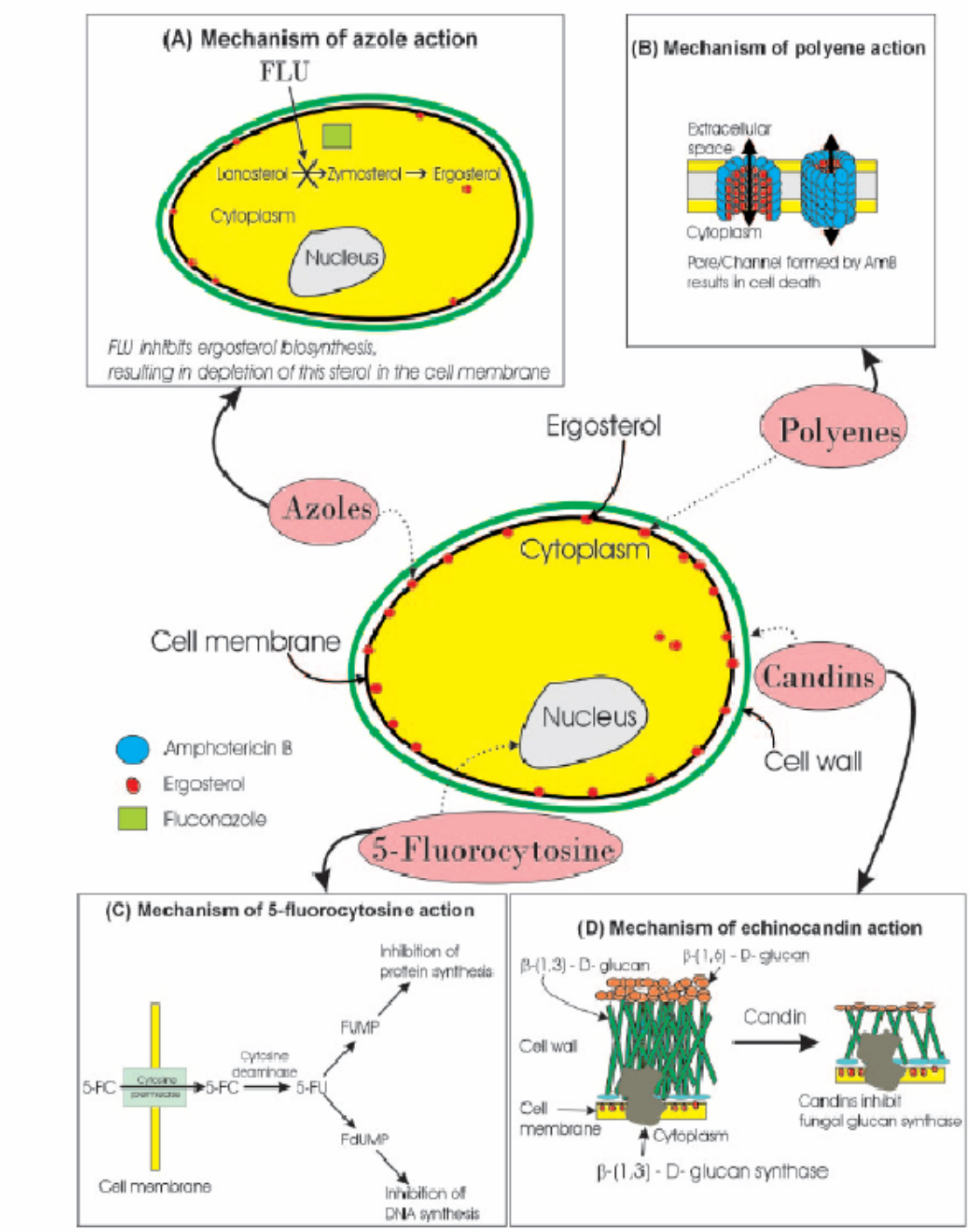

Figure1.17. Schematic description of sites of action of different antifungal agents.

Ergosterol biosynthetic pathway and the steps at which various antifungal agents exert their inhibitory activities are explained in figure 1-18.

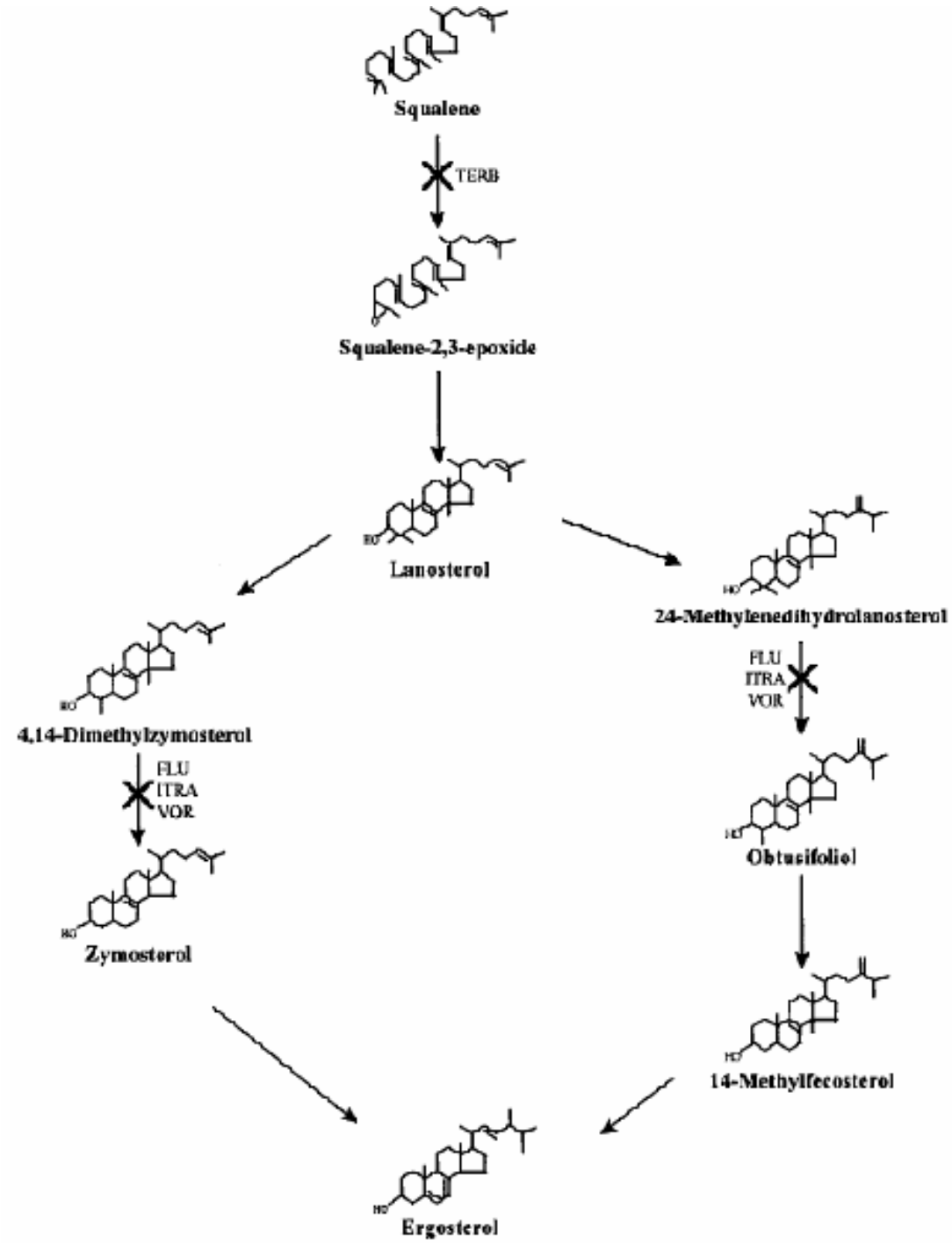

Figure1.18. Ergosterol biosynthetic pathway, steps at which various antifungal agents exert their inhibitory activities

The pharmacologic targets of different antifungal agents are illustrated in figure 1-19.

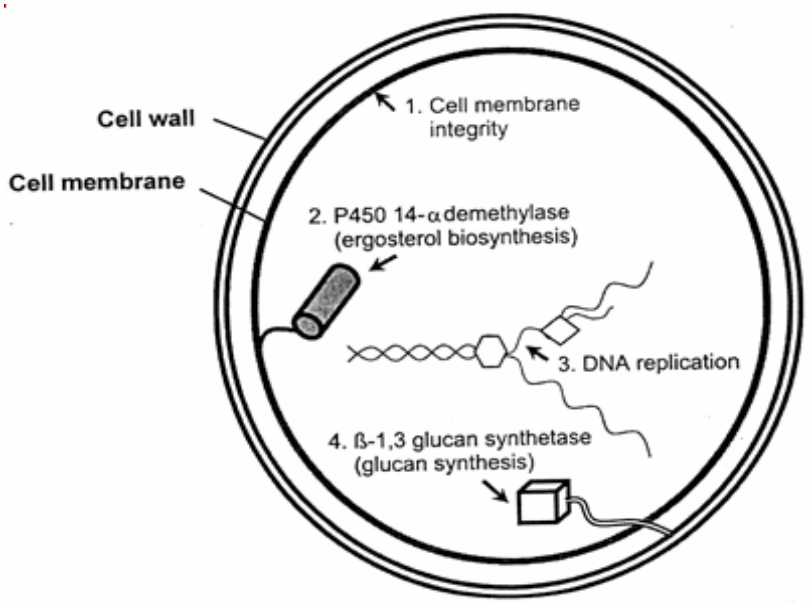

Figure1.19. Pharmacologic targets of antifungal agents

As has been previously mentioned, the antibiotics act by interfering with mitosis or, in the case of the polyenes, by binding to the sterol in the cell membrane, altering its permeability. These drugs are moderately effective, but are highly toxic to humans. Flucytosine is metabolized by the fungus into 5-flourouracil, which is then incorporated into RNA, disrupting DNA synthesis. This drug is rarely effective on its own as resistance emerges quickly; therefore, it is often combined with a polyene such as Amphotericin B. The azoles act by binding to the iron ion in cytochrome P-450, which inhibits the 14 α-demethylation oflanosterol, a step required for ergosterol and cholesterol synthesis. The synthetic Allylamines act similarly to the azoles in that they inhibit ergosterol synthesis, but they target an enzyme used in the synthesis of lanosterol. While these represent many effective topical and systemic drugs, the search still continues for safer and more effective antifungal agents.

1.14. Ketoconazole

1.14.1. Structure and chemistry

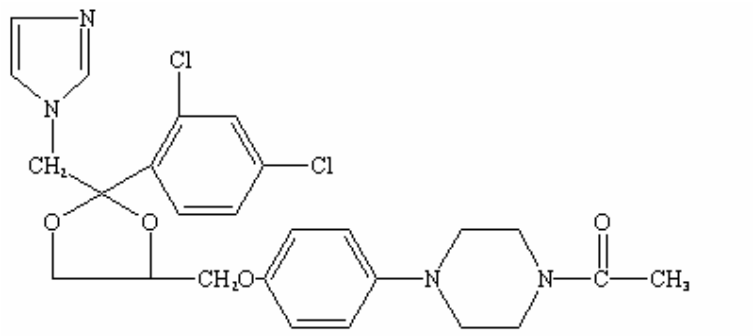

Figure1.20. Chemical structure of Ketoconazole

Ketoconazole is a synthetic azole antifungal agent an imidazole derivative (Piscitelli et al, 1991), which contains two nitrogen atoms in the five-membered azole ring (Cleary, et al., 1992) structurally related to other imidazoles e.g. butoconazole, clotrimazole, and econazole. Ketoconazole is cis-1-acetyl-4-[4-[[2-(2, 4-dichloro-phenyl) -2- (1H-imidazol-1-ylmethyl)-1, 3-dioxolan-4-yl] methoxyl] phenyl] piperazine (Cordoba-Diaz et al. 2001). The structural formula of ketoconazole is $C_{26}H_{28}C_{l2}N_4O_4$ shown in (Figure 1-20). It is a white to slightly beige, odorless powder, soluble in acids, with a molecular weight of 531.44 and a melting point of 148 - 152^0 C. Assay yields 98% - 102% (Anhydrous Basis). Ketoconazole as a dibasic compound has pK_a of 2.9 and 6.5. It is almost insoluble in water except at a pH lower than 3 (Daneshmend, 1981 and Daneshmend, 1990). Ketoconazole available as scored white tablets, each containing 200 mg ketoconazole base for oral administration. Inactive ingredients are colloidal silicon dioxide, cornstarch, lactose, magnesium stearate, microcrystalline cellulose, and povidone.

1.14.2. Mechanism of Action

Ketoconazole usually is fungistatic in action, but may be fungicidal at high concentrations after prolonged incubation or against very susceptible organisms (Sud and Feingold, 1981).

Like imidazoles, Ketoconazole presumably exerts its antifungal activity by altering cellular membranes resulting in increased membrane permeability, leakage of essential elements e.g.; amino acids, potassium, and impaired uptake of precursor molecules e.g.; purine and pyrimidine precursors to DNA. Although the exact mechanism of action of Ketoconazole has not been fully determined, it has been suggested that the fungistatic activity of the drug may result from interference with ergosterol synthesis, probably via inhibition of C-14 alpha demethylase, a microsomal cytochrome P-450 dependent enzyme system in susceptible fungi, which leads to accumulation of C-14 alpha methylated sterols e.g. lanosterol and decrease concentration of ergosterol. It appears that this may occur because the nitrogen atom in the drug binds to heme iron of cytochrome P-450 demethylase in susceptible fungi (Fabris et al, 1993, Matthew et al, 1993, Sud and Feinold, 1981 and Borgers, 1983). The depletion of ergosterol alters membrane fluidity, thereby reducing the activity of membrane-associated enzymes and leading to increased permeability and inhibition of cell growth and replication (Van den et al, 1983).

The fungicidal activity of the drug at high concentrations may result from direct physiochemical effect of the drug on the fungal cell membrane.

In Vitro, Ketoconazole concentration as low as 0.01 µg/ml prevents *Candida albicans* from forming pseudohyphae. This effect enhances phagocytosis of the fungi when polymorphonuclear leukocytes are added to the cultures because the Leukocytes can phagocytize yeast phase cells more easily than mycelial phase cells.

Further studies are needed to fully elucidate the effect of Ketoconazole on steroid synthesis in humans, but the drug apparently directly inhibits synthesis of adrenal steroids and testosterone in vitro and in vivo. Ketoconazole appears to inhibit steroid synthesis principally by blocking several P-450 enzyme systems e.g. 11 β-hydroxylase, C-17, 20-lyase; cholesterol side-chain cleavage enzyme.

Usual dosages (i.e., 200 – 400 mg daily) of ketoconazole have been reported to transiently (for 2 – 12 hours) inhibit testicular testosterone synthesis. A compensatory increase in serum luteinizing hormone (LH) concentrations may occur. Dosages of 800–1200 mg daily have been reported to have a more prolonged effect on testosterone synthesis. In a study in males receiving these high dosages, serum testosterone concentrations remained at a subnormal level (i.e., less than 300 ng/dL) throughout the day in about 30% of those receiving 800 mg daily and in all of those receiving 1200 mg daily. Oligospermia, decreased libido, and impotence often occurred in these males and azoospermia occurred rarely.

1.14.3. Spectrum

Ketoconazole is active against most fungi, including dermatophytes. The drug also has in vitro activity against some gram-positive bacteria, including *Staphylococcus aureus*, *Staphylococcus epidermidis*, *enterococci, Nocardia* and *Actinomadura*.

In vitro, Ketoconazole concentrations of 0.1 - 2 µg/ml generally inhibit most susceptible strains of *Blastomyces dermatitidis*, *Coccidioides immitis*, *Cryptococcus neoformans*, *Epidermophyton floccosum*, *Histoplasma capsulatum*, *Microsporum canis* and *Paracoccidioides brasiliensis* is generally inhibited in vitro by ketoconazole concentrations of 0.002 - 0.1 µg/ml (Borelli et al, 1979 and Como and Dismukes, 1994).

Ketoconazole concentrations of 1 - 25 µg/ml generally are required in vitro to inhibit *Actinomadura madurae*, *Aspergillus flavus*, *Nocardia species*, *petriellidium boydii* and *Sporothrix schenckii*.

A wide range of ketoconazole MIC values has been reported for Candida, MIC_{90} for *Candida albicans*, *Candida glabrata* and *Candida tropicalis* was 0.125 – 16 µg/ml.

1.14.4. Determination of ketoconazole in biological fluids

Pharmacokinetics studies and clinical trials of ketoconazole were limited due to a lack of readily available methods for quantitation of ketoconazole in serum or other biological fluids.

Several microbiological methods for the analysis of ketoconazole in plasma have been described.

Jorgensen et.al (1981) developed a bioassay for measurement of ketoconazole alone or in the presence of therapeutic levels of amphotericin B, using an agar diffusion assay incorporating Candida pseudotropicalis. Pairs of 8 – mm wells cut in the seeded assay medium were filled with four duplicate ketoconazole standards and duplicate patient specimens. Zones of inhibition were visible after 7 to 8 h of incubation, but were most easily measured after overnight growth. The assay allowed determinations of serum ketoconazole levels as low as 0.3 µg/ml with a 4.4% coefficient of variation. Thirty-five serum samples from patients receiving the drug were assayed by this method, and the results were compared with the Coccidioides immitis endospore assay. The correlation coefficient between the assays was 0.90. This assay was claimed to allow any microbiology laboratory to easily and safely determine ketoconazole levels in serum or cerebrospinal fluid.

Bodet and his coworkers (1986) developed an agar-well diffusion bioassay for measurement of flucytosine or ketoconazole using Candida pseudotropicalis ATCC 46764 as the assay organism. A test medium composed of (per liter) 7 g of Trypticase peptone, 7 g of YNB (yeast-nitrogen base), 15 g of glucose, and 15 g of agar was seeded with an inoculum, which had been grown to no. 2 McFarland turbidity after 4 to 6 h in YNB-glucose broth. Determinations of flucytosine or ketoconazole were performed without necessity of heating or diluting of serum samples to alleviate amphotericin B interference. A linear relationship between zone diameters and log10 concentration of the drug was observed over the pharmacologically relevant ranges of 25 to 160 µg /ml for flucytosine and 0.5 to 20 µg /ml for ketoconazole. The mean coefficient of variability for samples measured on 5 separate days was 2.4% for flucytosine and 4.0% for ketoconazole. This assay represents a significant improvement over previous bioassay methods

in that a single test system may be used for measurement of either flucytosine or ketoconazole, no serum dilution or pretreatment is required, inoculum preparation is accomplished entirely on the day of the assay, and sharp, clearly defined zones of inhibition are obtained with both drugs

The lack of specificity of the microbiological methods has led to the development of a number of procedures, which employ high-performance liquid chromatography (HPLC) for quantitative determination of ketoconazole in biological fluids.

A rapid and selective HPLC assay was described by Alton in 1980 as the first HPLC method for the quantitative determination of ketoconazole in human plasma. After extraction of the drug from plasma, the compound is separated by HPLC using a reversed-phase column and detected by UV light at 205 nm. Quantitation is accomplished by external standardization and the determination of peak areas is performed with the aid of an integrating computer. The average recovery of ketoconazole over a concentration range of 0.1 – 20 µg/ml was 88.2 ± 4.07% S.D. The maximum sensitivity of the assay is less than 0.1 µg/ml. The assay is suitable for use in pharmacokinetic studies following the administration of therapeutic doses of ketoconazole to humans.

Andrews et.al (1981) developed a reverse-phase, high-pressure liquid chromatographic method for the rapid and quantitative determination of ketoconazole. Drug levels from 0.5 to 10 µg/ml can be determined in either yeast nitrogen base medium or human serum by using an octadecylsilane column. A retention time of 4.9 ± 0.1 minutes resulted when the drug was eluted from a column with 75% methanol and 25% 0.02 M (pH 7.5) phosphate buffer at a flow rate of 2 ml/min. Optimum sensitivity was obtained at a wavelength of 231 nm.

In 1983 another HPLC procedure was described by Pascucci and associates for the quantitation of ketoconazole in biological fluids. The procedure involves sample preparation using a reverse-phase C_{18} cartridge prior to chromatography and quantitation using peak height ratios (UV absorbance detection, 231 nm) of ketoconazole to the internal standard, phenothiazine. A sensitivity of 0.2 µg/ml was achieved using a 0.5 ml sample. The mean recovery was 86.2%, and overall coefficient of variation of the

procedure was 7.1%. This procedure has been used to determine ketoconazole levels in human serum, plasma, CSF, and synovial fluid.

A reversed-phase high-performance liquid chromatographic (HPLC) method with terconazole as internal standard was described for the rapid analysis of ketoconazole in human serum. Except for diazepam no other drug interfered with the analysis. The lowest reproducible limit of the method was 0.05 mg/l. The coefficient of variation of the procedure was ≤ 7.5% over a range of ketoconazole concentrations from 1.0 to 10.0 mg/l and ≤ 16.75% over a range from 0.05 to 0.5 mg/l (Turner et al. 1986).

An accurate and precise procedure for the rapid analysis of ketoconazole in the lung, liver, plasma and adrenal gland of the rat has been developed by Riley and James (1986). Separation of the drug from endogenous substances was achieved by solid-phase extraction followed by reversed-phase chromatography on a Novapak C_{18} column using a mobile phase of methanol-acetonitrile-0.02 M phosphate buffer (pH 6.8) (35:30:35). Depending on the tissue, the recoveries of ketoconazole and clotrimazole, which was used as an internal standard, ranged between 85.0 to 93.6% and 79.3 to 84.1%, respectively? The reproducibility of the assay was between 3 and 4%, depending on the tissue involved. The sensitivity of the procedure permitted the monitoring of ketoconazole levels for up to 24 h in the adrenal and up to 48 h for plasma, lung and liver, following oral administration of 150 mg/kg of the drug to male rats.

An HPLC method using fluorescence detection instead of UV detection was developed by Yuen and Peh (1998) for the determination of ketoconazole in human plasma. The method entailed direct injection of the plasma sample after deproteinization using acetonitrile. The mobile phase comprised 0.05 M disodium hydrogen orthophosphate and acetonitrile (50:50, v/v) adjusted to pH 6. Analysis was run at a flow-rate of 1.5 ml/min with the detector operating at an excitation wavelength of 260 nm and an emission wavelength of 375 nm. The method is specific and sensitive with a quantification limit of approximately 60 ng/ml and a detection limit of 40 ng/ml at a signal-to-noise ratio of 3:1. Mean absolute recovery value was about 105%, while the within-day and between-day coefficient of variation and percent error values of the

assay method were all less than 14%. The calibration curve was linear over a concentration range of 62.5 – 8000 ng/ml.

Ramos et al. (2000) described three different approaches (methods A, B, and C) to attain high-throughput sample preparation and analysis in the quantification of ketoconazole in human plasma. Method A consisted of acetonitrile precipitation in a 96-well plate, transfer of the supernatant via a Tomtec Quadra 96 Model 320, and subsequent injection onto a 50 x 4.6 mm (i.d.) Develosil Combi-RP-5 column (packed with C_{30} bonded silica particles). Method B consisted of an identical sample preparation to method A with the exception that a Michrom Magic Bullet (trade mark) column, 2.0 - 0.50 mm (i.d., tapered bore) x 25 mm length, was used. Lastly, in method C, a turbulent-flow chromatography (TurboFlow LC/APCI-MS/MS) module was used for the direct analysis of ketoconazole in human plasma. A Sciex API 3000 was used in methods A and B, while a Micromass Quattro LC was employed in method C. Based on the values obtained for the calibrator (standard) and quality control samples, all three protocols yielded satisfactory accuracy, precision, and reduced manual sample preparation time.

Khashaba et.al (2000) suggested simple spectrophotometric and spectrofluorimetric methods for the determination of clotrimazole, econazole nitrate, ketoconazole, miconazole and tolnaftate. The spectrophotometric method depends on the interaction between imidazole antifungal drugs as n-electron donor with the p acceptor 2, 3-dichloro-5, 6-dicyano-1, 4-benzoquinone (DDQ) in methanol or with *p*-chloranilic acid (*p*-CA) in acetonitrile. The produced chromogens obey Beer's law at λ_{max} 460 and 520 nm in the concentration range 22.5 – 200 and 7.9 – 280 mg/ml for DDQ, and *p*-CA, respectively. The spectrofluorimetric method is based on the measurement of the native fluorescence of ketoconazole at 375 nm with excitation at 288 nm and or the induced fluorescence after alkaline hydrolysis of tolnaftate with 5 M NaOH solution at 420 nm with excitation at 344 nm. Fluorescence intensity versus concentration is linear for ketoconazole at 49.7 – 800 ng/ml while for tolnaftate; it is in the range of 20.4 – 400 ng/ml. The proposed methods were applied successfully for the determination of all the studied drugs in their pharmaceutical formulations.

Farhadi and Maleki (2001) proposed a spectrophotometric method for the determination of ketoconazole in pharmaceutical preparations. The method is based on the coupled redox-complexation reactions, which proceed in the ketoconazole-iron (III) and 1, 10-phenanthroline systems. A linear calibration graph was obtained between 1.6 – 16.0 ppm of ketoconazole. The procedure was successfully applied for the determination of Ketoconazole in tablet, cream and shampoo samples.

A high-performance liquid chromatographic assay with UV detection has been developed for the determination of ketoconazole in human plasma (Bruijn et al 2001). Quantitative extraction was achieved by a single solvent extraction involving a mixture of acetonitrile-n-butyl chloride (1:4, v/v). Ketoconazole and the internal standard (clotrimazole) were separated on a column packed with Inertsil ODS-80A material and a mobile phase composed of water-acetonitrile-tetrahydrofuran-ammonium hydroxide-triethylamine (45:50.2:2.5:0.1:0.1, v/v). The column effluent was monitored at a wavelength of 206 nm with a detector range set at 0.5. The calibration graph was linear in the range of 20 – 2000 ng/ml, with a lower limit of quantitation of 20.0 ng/ml. The extraction recoveries for ketoconazole and clotrimazole in human plasma were 93 ± 9.7% and 83 ± 10.0%, respectively. The developed method has been successfully applied to a clinical study to examine the pharmacokinetics of ketoconazole in a cancer patient.

A recent HPLC coupled with tandem mass spectrometry (LC–MS–MS) has been developed and validated for the determination of ketoconazole in human plasma. The method used diethyl ether to extract the ketoconazole and the internal standard (I.S.) R51012 from alkalinized plasma sample. The LC separation was on a C_{18} column (50×3 mm, 5 μm) using acetonitrile–water–formic acid (75:25:1, v/v/v) mobile phase. The retention times were approximately 1.8 minute for both ketoconazole and the I.S. The MS–MS detection was by monitoring 531.2→82.1 (*m*/*z*) for ketoconazole, and 733.5→460.2 (*m*/*z*) for the I.S. The dynamic range was from 20.0 to 10000 ng/ml based on 0.1 ml plasma, with linear correlation coefficient of ≥ 0.9985. The run time was 2.5 minute/injection. The recoveries of ketoconazole and the I.S. were 102 and 106%, respectively. The precision and accuracy of the control samples were with the relative standard deviations (RSDs) of ≤ 4.4%

(n=6) and the relative errors (REs) from −0.6 to 1.4% for intra-day assay, and ≤ 8.6% RSD (n=18) and −1.4 to 0.9% RE for inter-day assay. The partial volume tests demonstrated good dilution integrity. Three freeze–thaw cycles, keeping plasma samples at ambient for 24 hours, storing extracted samples at ambient for 24 h, and storing frozen plasma samples at approximately −20° C for up to 2 months did not show substantial effects (Chen et al, 2002).

1.14.5. Pharmacokinetics

Pharmacokinetics involves the kinetics of drug absorption, distribution, and elimination (i.e. excretion and metabolism). The study of pharmacokinetic involves both experimental and theoretical approaches. The experimental aspect of pharmacokinetics involves the development of biological sampling techniques, analytical methods for measurement of drugs and metabolites, and procedures that facilitate data collection and manipulation. The theoretical aspect of pharmacokinetic involves the development of pharmacokinetic models that predict drug disposition after drug administration. The application of statistics is an integral part of pharmacokinetics studies. Statistical methods are used for pharmacokinetic parameters estimation and data interpretation. Statistical methods are applied to pharmacokinetic models to determine data error and structural model deviations. Mathematics and computer techniques form the theoretical basis of many pharmacokinetics methods (Shargel and Andrew, 1999).

1.14.5.1. Pharmacokinetics parameters

a) Elimination rate constant

The rate of elimination (ke) for most drugs is a first-order process. The elimination rate constant, k, is a first-order elimination rate constant with units of time^{-1} (e.g. hr^{-1}).

b) Biological half-life

The half-life ($t_{½}$) is the time required for the concentration to become equal to one-half of the initial concentration.

c) Apparent volume of distribution

The volume of distribution (Vd) represents a volume that must be considered in estimating amount of drug in the body from the concentration of drug found in the sampling compartment. The volume of distribution is also the apparent volume in which the drug is dissolved.

e) Clearance

Clearance (Cl) is the measure of drug elimination from the body without identifying the mechanism or process.

f) Mean residence time

The term means residence time (MRT) describes the average time for all the drug molecule to reside in the body. MRT may be considered also as the mean transit time (Notari et.al, 1975).

1.14.5.2. Bioavailability

Bioavailability indicates a measurement of the rate and extent (amount) of therapeutically active drug that reaches the systemic circulation and is available at the site of action (FDA, 2003).

1.14.5.3. Bioavailability parameters

a) Maximum plasma concentration (C_{max})

The peak plasma drug concentration represents the maximum plasma drug concentration obtained after oral administration of drug. C_{max} provides indications that the drug is sufficiently systemically absorbed to provide a therapeutic response. In addition, C_{max} provides warning of possibly toxic levels of drug.

b) Time to reach C_{max} (T_{max})

The time of peak plasma concentration (T_{max}) corresponds to the time required to reach maximum drug concentration after drug administration. At T_{max} peak drug absorption occurs and the rate of drug absorption exactly equals the rate of drug elimination. Drug absorption still continues after T_{max} is

reached, but at slower rate. When comparing drug products, T_{max} can be used as an approximate indication of drug absorption rate.

c) Area under plasma concentration-time curve (AUC)

It is the measurement of the extent of drug bioavailability. For many drugs the AUC is directly proportional to dose. In some cases the AUC is not directly proportional to the administered dose for all dosage levels. These types of drugs are described to have non-linear pharmacokinetics behavior (Shargel and Andrew, 1999).

1.14.5.4. Methods for assessing bioavailability

Direct and indirect methods may be used to assess drug bioavailability. The design of bioavailability study depends on the objectives of the study, the ability to analyze the drug (and metabolites) in biological fluids, the pharmacodynamic of the drug substance, the route of drug administration and the nature of the drug product. Pharmacokinetic and/or pharmacodynamic parameters as well as clinical observations and in vitro studies may be used to determine drug bioavailability from a drug product.

a) Absolute bioavailability

The absolute bioavailability of a given drug from a dosage form is the fraction or (percentage) of the administered dose which is absorbed intact into the systemic circulation.

For equivalent doses of administration drug:

$$\text{Absolute bioavailability} = \frac{(AUC_{\infty})_{abs}}{(AUC_{\infty})_{iv}}$$

Where $(AUC_{\infty})_{abs}$ is the total area under the plasma concentration-time curve following the administration of a single dose via an absorption site and $(AUC_{\infty})_{iv}$ is the total area under the plasma concentration-time curve following administration by rapid intravenous injection.

For non-equivalent doses of administration drug:

$$\text{Absolute bioavailability} = \frac{(AUC_{\infty})_{abs} \text{ X } D_{iv}}{(AUC_{\infty})_{iv} \text{ X } D_{abs}}$$

Where D_{abs} is the size of the single dose of drug administered via the absorption site, and D_{iv} is the size of the dose of drug administered as an intravenous bolus Injection.

The absolute bioavailability of a given drug from particular type of dosage form may be expressed as a fraction or more commonly, as a percentage.

b) Relative bioavailability

In the case of drugs that cannot be administered by intravenous bolus injection, the relatives (or comparative) bioavailability is determined rather than the absolute bioavailability. In this case the bioavailability of a given drug from a “test” dosage form is compared to that of the same drug administered in “standard” dosage form, which is either an orally administered solution from which the drug is known to be well absorbed or an established commercial preparation of proven clinical effectiveness.

$$\text{Relative bioavailability} = \frac{(AUC_{\infty})_{test}}{(AUC_{\infty})_{standard}}$$

Where $(AUC_{\infty})_{test}$ and $(AUC_{\infty})_{standard}$ are the total areas under the plasma concentration-time curves following the administration of a single dose of the test dosage form and of the standard dosage form, respectively.

When different doses of the test and standard dosage forms are administered, a correction for the size of dose is made as follows:

$$\text{Relative bioavailability} = \frac{(AUC_{\infty})_{test} \text{ X } D_{standard}}{(AUC_{\infty})_{standard} \text{ X } D_{test}}$$

Where D_{test} and $D_{standard}$ are the sizes of the single doses of the test and standard dosage forms, respectively.

Both absolute bioavailability and relative bioavailability may be expressed as a fraction or as a percentage (Shargel and Andrew, 1999).

1.14.5.5. Factors affecting drug absorptions from gastrointestinal tract

Factors that affect drug absorption from gastrointestinal tract include gastric emptying, gastrointestinal pH, the environment within the lumen, influence of food in the gastrointestinal tract, disease state and physiological disorders and the mechanisms of transport of the drugs across the gastrointestinal membrane (Aulton, 2002).

1.14.5.6. Pharmaceutical factors affecting drug bioavailability

The formulation factors affecting drug bioavailability can be summarized in the following:

a) Disintegration

Release of the drug from the dosage form.

b) Dissolution

Factors that affect drug dissolution of a solid oral dosage form include (a) the physical and chemical nature of active drug substance, (b) the nature of the ingredients, and (c) the method of manufacture (Shargel and Andrew, 1999).

1.14.6. Pharmacokinetics of Ketoconazole

1.14.6.1. Absorption

Ketoconazole is rapidly absorbed from gastrointestinal tract (Fabris et al, 1993). In healthy, fasting adults, oral bioavailability of the drug is similar following administration of conventional tablets or suspension but is somewhat higher following administration of solution (Baxter, 1986). Oral

absorption of ketoconazole varies among individuals and bioavailability of tablet is 75% (Koch, 1983; Graybill and Drutz, 1980 and Chambers, 2001).

The bioavailability of oral Ketoconazole depends on the pH of gastric contents in the stomach. An increase in the pH results in decrease absorption of the drug.

Concomitant administration of acidic beverages may increase bioavailability of oral Ketoconazole in some individuals with achlorhydria. In one cross-over study in healthy, fasting adults with achlorhydria (induced by administration of 60 mg of oral omeprazole 6–8 hours prior to ketoconazole), the peak plasma concentration and area under the plasma concentration-time curve (AUC) of the drug averaged 0.8 mcg/mL and 3.46 mcg/mL per hour, respectively, when the dose was administered with 240 mL of water and averaged 2.44 mcg/mL and 11.22 mcg/mL per hour, respectively, when the dose was administered with 240 mL of Coca-Cola Classic (pH 2.5). Concomitant administration of drugs, which increase gastric pH, may decrease absorption of ketoconazole (Chin, 1995).

The effect of food on the rate and extent of gastrointestinal tract absorption of Ketoconazole has not been clearly determined. However the administration of the drug with food increases the extent of absorption and results in more consistent plasma concentration of Ketoconazole (Daneshmend, 1991) .The food increase absorption by increasing the rate and/or extent of dissolution of Ketoconazole e.g.; by increasing bile secretions or by delaying stomach emptying.

In healthy, fasting adults, peak plasma ketoconazole concentrations of approximately 4.2, 5, or 6.2 µg/mL occurred 1 – 2 hours following oral administration of a single 200 mg dose as tablets, a suspension, or a solution, respectively. Mean elimination half-life range from 7.5 – 7.9 hour (Huang et al, 1986). This dose when given to non fasting in other study, peak plasma concentrations of the drug attained within 1 – 4 hours and range form 1.5 – 4.5 µg/ml; plasma concentrations of the drug were usually less than 0.05 µg/ml after 24 hours.

Considerable interindividual variations in peak plasma concentrations attained and areas under the concentration–time curves (AUCs) have been reported with a specific oral dose of ketoconazole. In one cross-over study in

adults who received single oral doses of ketoconazole of 100, 200 and 400 mg a comparison of dose versus AUC suggested that Ketoconazole undergoes saturable first pass elimination since bioavailability of the lower dose was relatively poor (Daneshmend, 1981).

1.14.6.2. Distribution

Ketoconazole has been detected in urine, bile, saliva, synovial fluids and cerebrospinal fluids following oral administration of a single 200 mg dose of the drug in adults (Heel, 1982). Central nerves system penetration of the drug is unpredictable and has generally been considered to be minimal.

In rats, highest concentrations of ketoconazole are attained in the liver, pituitary and adrenals; moderate concentrations are attained in the lungs, kidneys, bladder, bone marrow, teeth, myocardium and various glandular tissues; and lowest concentrations are attained in the brain and testes following a single oral dose of the drug (Matthew et al, 1993). It is not known if ketoconazole cross the placenta in human; however, the drug crosses the placenta in rats. Ketoconazole probably distributes into human milk. Ketoconazole is 84 – 99% bound to plasma protein primarily albumin (Fabris et al, 1993 and Chambers, 2001), but unbound drug distributes well throughout most tissues. Because of their lipophilicity, the concentrations of ketoconazole, in urine and cerebrospinal fluid are typically less than 1.0 µg per milliliter (Daneshmend and Warnock, 1988). Plasma protein binding of ketoconazole was altered in patients with chronic renal and hepatic cirrhosis, with the percentage of free ketoconazole markedly increased compared to controls (Martinez-Jorda et al, 1990).

1.14.6.3. Elimination

Plasma concentrations of Ketoconazole appear to decline in biphasic manner with half-life of approximately 2 hours in the initial phase and approximately 8 hours in the terminal phase. A half-life of 1 hour has been observed in immunocompromised patients, and a half-life of 11.6 hours has

been observed in cancer patients (Daneshmend and Warnock, 1988, Badcock et al, 1987).

Ketoconazole is partially metabolized, in the liver to several inactive metabolites by oxidation and degradation of the imidazole and piperazine rings, by oxidative O-dealkylation and by aromatic hydroxylation to a large number of metabolites, which do not possess antifungal activity (Daneshmend, 1981). Urinary excretion of ketoconazole account for only 2 – 3% (Fabris, 1993). The major route of elimination of ketoconazole and its metabolites appears to be excretion into the feces via the bile. The hepatic clearance of ketoconazole is saturable such that AUC increases disproportionally with increasing dose (Matthew et al, 1993 and Daneshmend et al, 1984).

In one study in fasting adults with normal renal function, approximately 57% of a single 200 mg oral dose of ketoconazole was excreted in the faces within 4 days: 20 – 65% of this was unchanged drug. In the same study, approximately 13% of the dose was excreted in urine within 4 days; 2 – 4% of this was unchanged drug (Graybill and Drutz, 1980). Ketoconazole is not appear to be dialyzable (Brass et al, 1982)

1.14.7. Uses of ketoconazole

Oral Ketoconazole can be used in the treatment of the following:

1.14.7.1. Blastomycosis

Blastomycosis caused by *Blastomyces dermatitidis*, as alternative for the treatment of mild to moderate disease. The recommended dose is 200 mg once daily and may be increased to 400 mg once daily for serious infections. In a multi-center prospective study, high oral doses of 800 mg daily were found more effective than lower oral doses of 400 mg daily in the treatment of blastomycosis. Success rate after six months of treatment were 100% with high doses and 79% with low dose therapy (Anon, 1985). Ketoconazole, at a dose of 400 to 800 mg daily, is effective in 70 to 100 percent of patients (Como and Dismukes, 1994). For patients who are immunocompromised such as transplanted patients or patients with AIDS, treatment with amphotericin B followed by long-term treatment with ketoconazole in doses of

400 to 800 milligrams daily has been used (Serody, et al., 1993 and Pappas, et al., 1992)

1.14.7.2. Candidiasis

a) Mucocutaneous candidiasis: The recommended dose is 200 mg once daily and may be increased to 400 mg once daily if clinical response is not sufficient. Recommended treatment duration for candidiasis is one or two weeks. The long-term efficacy of oral ketoconazole in thirteen patients with chronic mucocutaneous candidiasis has been evaluated (Mobacken and Moberg, 1986). In an open noncomparative trial, thirteen patients received 200 mg ketoconazole once daily for fourteen months. Therapy was continued until clinical resolution of symptoms and negative candida culture were obtained. On the average, oral lesions resolved after 1 – 2 months, skin lesions after two months, finger paronychia after 3 – 4 months and nail lesions after 4 – 14 months. Ketoconazole was reported to be effective in treating chronic mucocutaneous candidiasis in dose of 100 to 400 mg daily for 3 – 6 months (Drouhet and Dupont, 1980 and Hay et al, 1980).

b) Oral candidiasis: Ketoconazole 200 – 800 mg daily for 2 – 3 weeks has been found useful for treating severe oral or esophageal candidiasis in patients with the acquired immunodeficiency syndrome (AIDS) (Fauci et al, 1984 and Anon, 1991).

c) Uncomplicated vulvovaginal candidiasis in non-pregnant women: The usual dose of Ketoconazole is 200 – 400 mg twice daily for 5 days. (Talbot and Spencer, 1983; Kovacs, et al., 1990 and Sobel, 1986).

1.14.7.3. Chromomycosis

The usual oral dose for adult is 200 – 400 mg. Treatment should be continued for a minimum of 6 months (Prod Info Nizoral®, 1995)

1.14.7.4. Coccidioidomycosis

The availability of the antifungal azoles has changed the outlook for the management of coccidioidomycosis, long considered one of the most refractory of the systemic mycoses to therapy. Ketoconazole in 200 – 400 mg

daily for 2 – 11 months has proven effective in coccidioidomycosis (Graybill et al, 1980 and Brass et al, 1980). Twenty-nine patients in an uncontrolled study were given 200 mg/day for more than three months before increasing their dose. Only 16% of patients responded to 200 mg/day. Seventeen of these patients had their dose increased to 400 or 800 mg/day and 71% responded to treatment. This data strongly indicates that 200 mg/day is not an adequate dose for treating systemic mycosal diseases such as coccidioidomycosis and histoplasmosis (Anon, 1982).

The Medical Literature recommends either ketoconazole or amphotericin B as drugs of choice to treat Coccidioidomycosis infections. The recommended dose of ketoconazole is 400 mg daily (Anon, 1992).

1.14.7.5. Histoplasmosis

A low dose of 400 mg/day was found more effective than a higher dose of 800 mg/day in patients with localized or disseminated histoplasmosis treated for six months or longer, success rate was 100% and 57%, respectively. In patients with progressive disease during the first month of treatment, the dose increases to 600 or 800 mg/day (Anon, 1985).

1.14.7.6. Paracoccidioidomycosis

Doses of 200 – 400 mg daily for a period of 2 – 19 months have proven effective in paracoccidioidomycosis (Negroni et al, 1980 and Restrepo et al, 1980).

1.14.7.7. Leishmaniasis

Ketoconazole doses of 400 – 600 mg daily for 4 – 8 weeks has been found useful for treatment of Cutaneous, mucocutaneous or visceral leishmaniasis

1.14.7.8. Pityriasis (Tinea) versicolor

A dose of 200 mg/day for five days is considered effective for most cases of Pityriasis versicolor (Hay and Midgeley, 1984).

1.14.7.9. Eumycetoma

A dose was 200- 400 mg daily for periods ranging from three months to three years was found to be effective in the treatment of eumycetoma caused by *Madurella mycetomatis*. (Mahgoub and Gumaa, 1984).

1.14.7.10. Transplantation

A Ketoconazole dose of 82 mg/day is required for reduce cyclosporine dose by 73% at twelve moths and 70% at fifty-four months of concurrent use of ketoconazole and cyclosporine in kidney transplant recipients (Sobh et al, 2001).

1.14.7.11. Renal failure

Dose reduction is not required, since very little active drug is excreted via the kidneys (Graybill and Drutz, 1980).

1.14.7.12. Hepatic insufficiency

Ketoconazole is extensively metabolized in the liver. However, specific dosing adjustments have not been described (Graybill and Drutz, 1980). Dose reductions should be considered in patients with severe liver disease.

1.14.8. Adverse effects of Ketoconazole

As ketoconazole is one of azole compounds, a number of side effects are associated with ketoconazole as a result of inhibition of mammalian enzymes (Venkatakrishnan, 2000).

a) ***Gastrointestinal tract effects:*** The most frequent adverse effects of ketoconazole are nausea and/or vomiting which have been reported in 3 - 10% of patients receiving 400 mg/day but increase to more than 50% in patients receiving more than 800 mg/day (Chambers, 2001 and Sugar, et al., 1987). Abdominal pain, a diverse gastrointestinal tract effect, appears to be dose related. These side effects are reported less frequently when ketoconazole is administered with food, and usually subside with continued therapy with the drug.

b) ***Hepatic effects:*** Ketoconazole leads to liver damage due to its ability to inhibit cytochrome P450 3A4, the major P450 isoform of the liver (Suzuki, 2000). The inhibition of P450 3A4 results in drug-drug interactions involving ketoconazole and a decrease in the rate of clearance of many drugs (Tsunoda, 1999). Steroid biosynthesis by P450 enzymes is also inhibited by ketoconazole, presumably due to the binding of ketoconazole to the mitochondria of P450 enzymes, the drug causes transient increase in serum AST (SGOT) and ALT (SGPT). Ketoconazole induced hepatotoxicity usually is reversible following discontinuance of the drug.

c) ***Endocrine effects:*** administration of low doses of ketoconazole leads to a significant reduction in serum androgen levels (Sikka, 1985). Bilateral gynecomastia with breast tenderness has occurred in some men during therapy with Ketoconazole.

d) ***Other Adverse effects:*** Pruritus in about 2%, Rash, dermatitis and purpura, headache, dizziness, arthralgia, fever and Chills (Chambers, 2001).

1.14.9. Precautions and contraindications

Ketoconazole has been associated with hepatotoxicity, which rarely results in death.

Patients receiving the drug should be closely monitored clinically and biochemically. Liver function tests, including alkaline phosphatase, GGT, GGTP and bilirubin, should be performed prior to initiation of Ketoconazole therapy and frequently (e.g.; biweekly during the first two months of therapy and monthly or bimonthly thereafter) during therapy, particularly in patients receiving prolonged therapy or other potentially hepatotoxic drugs In patients with a history of hepatic disease the possibility that Ketoconazole may depress adrenocortical function should be considered.

Ketoconazole is contraindicated in patients with Known hypersensitivity to the drug. Concomitant administration of Ketoconazole and terfenadine or astemizole is contraindicated (Prod Info Nizoral®, 1998).

1.14.10. Mutagenicity and carcinogenicity

There was no evidence of Ketoconazole–induced carcinogenicity or mutagenicity in long term study in mice and rats. Its use during pregnancy is not recommended, and because of secretion of the drug into breast milk, its use in nursing mothers also is unwise (Chambers, 2001).

1.14.11. Drug interactions

- Concomitant administration of drugs, which decrease gastric acid output or increase gastric pH, may decrease absorption of the antifungal agent. (Brass, et al., 1982 and Carison, et al., 1983).
- Concomitant administration of Ketoconazole and rifampin has resulted in decrease serum concentrations of Ketoconazole.
- Norfloxacin may enhance the antifungal activity of antifungal agents.
- Concomitant administration of Ketoconazole and cisapride is contraindicated also with terfenadine and astemizole .
- Like other imidazole derivatives Ketoconazole may enhance the anticoagulant effect of cumarin drugs (Brass et al. 1982).
- Concomitant administration of Ketoconazole with cyclosporine, tacrolimus, corticosteroids and midazolam may result in increased plasma concentration of this drug (Abraham et al.2003). However, ketoconazole is less expensive than fluconazole and itraconazole, an especially important consideration for patients receiving long-term therapy.

1.15. Clinical Studies

The pharmacology of ketoconazole in patients with fungal infections after the administration of 50, 100, and 200 mg doses of ketoconazole was studied by Brass et al. (1982). They observed that there was a linear increase in the area under the serum concentrations curve; which was not apparent when higher doses of ketoconazole were given. An increase in the area under the curve occurred in patients receiving 200 mg daily who were restudied after 1 to 12 months of therapy. However, normalized area under the curve appeared to decrease after higher doses were administered chronically. The half life ranged from 2.0 to 3.3 hours. Peak serum concentrations up to 50 µg/ml were detected in this study, and potentially therapeutic concentrations were detectable up to 26 hours after high doses. Ketoconazole penetrated the saliva and inflamed joint fluid and meninges, although variably, and could be demonstrated in some other tissue compartments. In the presence of renal failure, ketoconazole disposition was not altered, whereas in the presence of hepatic insufficiency, an alteration in disposition was suggested. The interactions of ketoconazole and other drugs were studied. Of note, antacids did not significantly affect ketoconazole pharmacokinetics (nor did meals), and ketoconazole and warfarin did not appear to affect the pharmacokinetics of the other.

Single oral doses of ketoconazole, 200 mg or miconazole, 250 mg were given in a randomized cross-over study to 10 healthy volunteers. Ketoconazole was administered (i) after fasting (both brand 1 [Orion Pharmaceutical Co.] and brand 2 [Janssen Pharmaceutical] were tested), (ii) after a standardized meal (660 Kilocalories; 2,772 kJ) (brand 1), and (iii) with 300 ml of orange juice (pH 3.8) (brand 1). Miconazole was administered after fasting. Venous blood samples for HPLC determinations of ketoconazole and gas chromatographic (GC) analyses of miconazole were drawn periodically up to 24 hours. The concentrations of ketoconazole in sera attained with the two brands were not statistically different. The peak concentrations of ketoconazole attained with brand 1 were 4.1 ± 0.3 µg/ml (mean ± standard error of the mean) after fasting, 2.3 ± 0.3 µg/ml after the standardized meal (P

< 0.01), and 3.6 ± 0.2 μg/ml with orange juice. The peak concentrations were reached in 1.4, 2.3 (P < 0.05), and 1.8 hours, respectively, whereas the areas under the serum concentration-time curves were 14.4 ± 2.21, 8.6 ± 1.33 (P < 0.05), and 13.4 ± 1.30 μg.h/ml, respectively. The half-lives (1.7 to 2 hours) did not vary significantly among the different regimens. Compared with ketoconazole, oral absorption of miconazole was poor (peak concentration, 0.47 ± 0.7 μg/ml; time to reach the peak concentration, 2.6 hours; area under the serum concentration-time curve, 1.10 ± 0.20 μg.h/ml) (Mannisto, 1982).

In a study involving patients with advanced malignancies, twenty-seven volunteers were given 200 mg of ketoconazole orally every 6 or 12 hours. Blood samples were collected during these intervals and after the last dose to determine plasma concentrations and half-lives (Maksymiuk, 1982). The mean plasma concentrations measured after the initial dose were 1.7 ± 1.1 μg/ml at 2 hours, 0.9 ± 0.2 μg/ml at 6 hours, and 0.7 ± 0.4, μg/ml at 8 hours. Plasma concentrations rose significantly in patients on the every-6-hours schedule. Concentrations were more variable in patients on the every-12-hours schedule, and changes in mean plasma concentrations after 7 and 14 days were not significant. Half-lives ranged from 1.3 to 11.6 hours in individual patients. The mean half-life for all patients studied was 3.7 ± 0.6 hours on day 1. The calculated area under the curve was 12.0 ± 4.7, μg.h/ml on day 1; it increased after 7 and 14 days of administration (every-6-hours schedule), suggesting plasma binding or wide drug distribution or both. Saturation of storage compartments is also suggested. Less than 1% of the administered dose was recoverable active drug from the urine over 6 hours.

Daneshmend and his coworkers (1983) investigated the pharmacokinetics of Ketoconazole in healthy subjects. Ketoconazole 200 mg was given twice daily for five days to eight subjects. The serum pharmacokinetics was examined with the first dose on day 1 and with the ninth dose on day 5. The serum half-life of ketoconazole was 74.8 ± 13.9 minutes (mean ± S.D.) on day 1, and 155.9 ± 32.7 minutes on day 5. On day 1 the area under the serum concentration time curve was 626.9 ± 230.1 mg/l.min, and 1441 ± 897.3 mg/l.min on day 5. Antipyrine kinetics was determined before and after the course of ketoconazole. There was no significant change in the clearance and half-life of antipyrine. Ketoconazole in

the doses used in this study does not appear to alter hepatic microsomal enzyme activity in man. The increased ketoconazole half-life and area under the serum concentration-time curve on day 5 were in part caused by food. Other mechanisms for these changes such as saturation of a tissue compartment or a change in the elimination of ketoconazole during chronic dosing may also be responsible.

Delivery of ketoconazole to human stratum corneum was studied by Harris associates (1983). Thirteen healthy volunteers, three patients with chronic fungal disease and one patient with palmar-plantar hyperhidrosis were given 400 mg of ketoconazole daily for various lengths of time. The ketoconazole content of palmar stratum corneum, eccrine sweat, sebum, and serum was measured by HPLC (sensitivity, 0.005 to 0.010 μg/ml). Palmar stratum corneum obtained after 7 and 14 days of daily administration contained up to 14 μg of ketoconazole per g. Ketoconazole was not found in sebum after 7 or 14 days of daily ingestion of the antimycotic agent. Sebum from three patients with chronic fungal infection treated for > 9 months contained ketoconazole (x, 4.7 pg/g). Thermogenic whole body eccrine sweat contained a mean of 0.059 pg/ml on day 7 and 0.084 pg/ml on day 14 of daily administration. Ketoconazole appeared in Thermogenic whole body eccrine sweat and palmar hyperhidrotic sweat within 1 hour after a single oral dose. Partition studies of ketoconazole containing eccrine sweat demonstrated a 10-fold greater concentration in the sediment phase (desquamated keratinocytes) compared with the clear supernatant phase. In vitro studies with [^{3}H] ketoconazole-supplemented supernatant sweat revealed preferential binding to stratum corneum, hair, and nails and its partitioning to lipid-rich sebum. The study concluded that eccrine sweat rapidly transports ketoconazole across the blood-skin barrier, where it may bind or partition to keratinocytes and surface lipids.

Daneshmend and his coworkers (1984) studied the Influence of Food on the pharmacokinetics of Ketoconazole. Eight healthy adults were given single oral doses of ketoconazole (200, 400, 600, and 800 mg) in the fasting State and with a standard breakfast at weekly intervals according to a balanced block design. Concentrations in serum were measured up to 32 h after the dose. Food did not reduce ketoconazole absorption or significantly

alter peak ketoconazole concentrations in serum, though there was a food-related delay in achieving peak concentrations. At the 400 and 600 mg doses, food appeared to enhance absorption, but this effect was not seen at the 800 mg dose. With an increase in dose, the half-life and area under the serum concentration-time curve increased disproportionately, suggesting that the pharmacokinetics of ketoconazole may be dose dependent. Up to the 800 mg dose, the elimination of ketoconazole did not appear to be saturable. Administration of the drug with food is unlikely to be a cause of therapeutic failure.

It has been emphasized that Ketoconazole inhibits adrenal steroidogenesis in normal subjects during short term treatment. Since this drug is used in the long term prophylaxis of fungal infections in patients with hematological malignancies, Dandona and associates (1985) have investigated whether such patients have evidence of adrenocortical suppression. Six patients on long term prophylaxis with ketoconazole were given tetracosactrin stimulation tests. All patients had high basal cortisol concentrations, which increased further after administration of 25 IU of tetracosactrin. It is concluded that leukaemic patients receiving long term Ketoconazole treatment do not have adrenocortical suppression. They may, in fact, have hyperstimulated adrenocortical function due to stress and hypovolemia.

The pharmacokinetics and bioavailability of ketoconazole given as a 200 mg single dose in a tablet, suspension, or solution were studied in 24 fasting healthy males by using a crossover design (Huang et al, 1986). Levels of ketoconazole in plasma were determined for up to 48 hours by a sensitive reverse-phase HPLC method. The absorption of ketoconazole was rapid, with mean maximum concentrations of the drug in plasma of 4.2, 5.0, and 6.2 µg/ml attained at 1.7, 1.2, and 1.0 hours, respectively, after administration of the tablet, suspension, and solution, respectively. The mean distribution and elimination half-life values were 1.5 to 1.7 and 7.5 to 7.9 h, respectively. The mean oral clearance of the solution dose was 209 (± 82.9 standard deviation) ml/min, and the mean apparent volume of distribution was 88.31 (± 68.72) liters. The relative bioavailabilities for the tablet and suspension were 81.2 (± 33.5) and 89.0 (± 23.1)%, respectively, of that of the solution. The data

indicated the bioequivalence of the tablet to the suspension and of the suspension to the solution. Dose proportionality of ketoconazole was also studied in 12 volunteers after they received solution doses of 200, 400, and 800 mg. Linear correlations between the dose and the maximum concentration of the drug in plasma, the time to the maximum concentration, and the area under the concentration-time curve were observed. However, the increase in the area under the curve was more than proportional to the dose given. The levels in plasma seemed to decay at a lower rate after 400 and 800 mg doses. The mean oral clearance decreased from 244.9 to 123.6 and 80.0 ml/min, respectively, as the dose increased from 200 to 400 and 800 mg. The apparent dose-dependent kinetics may have been due to the presystemic elimination and capacity-limited hepatic metabolism, which become saturated at higher doses.

Ketoconazole pharmacokinetics were determined in nine adults with hematological malignancy after one week on a 200 mg daily dose and later after one week on a 400 mg daily dose. The area under the serum concentration-time curve (AUC) reached 12.3 ± 7.7 mg/l.h (mean ± S.D.) on the 200 mg dose and increased to 23.0 ± 18.2 mg/l.h on the 400 mg dose ($p < 0.05$). The half-life of ketoconazole was 3.1 ± 1.9 h on the 200 mg dose and 3.5 ± 1.7 h on the 400 mg dose. Peak concentrations were 3.2 ± 1.8 mg/l and 4.6 ± 3.2 mg/l on the 200 mg and 400 mg doses, respectively. Trough ketoconazole concentrations were undetectable 24 hours after either dose. There was no correlation between the leukocyte count and any pharmacokinetic parameter for ketoconazole. Variation in AUC was 20-fold on the 200 mg daily dose and 8-fold on the 400 mg per day regimen. Measurement of serum levels during ketoconazole treatment appears necessary in view of the unpredictable concentrations achieved. Once-a-day dosage regimens of Ketoconazole in immunocompromised patients may be inappropriate. The investigators recommended that future clinical trials should adopt a two or three times a day dosing regimen, as this may confer a pharmacokinetic and therapeutic advantage (Stockley et al., 1986).

Biliary excretion, and pharmacokinetics of ketoconazole in Sprague-Dawley rats were determined after intravenous administration (Remmel, 1987). Greater than 80% of the radioactivity after a 5 mg/kg IV dose of ^{3}H-

ketoconazole was excreted in the feces. Urinary excretion was essentially complete after 48 hours; however, fecal excretion was prolonged over a 7-day period. Biliary excretion of radioactivity averaged 54.3 ± 18.0% of the dose over a 7.5 – 8 hour period in pentobarbital-anesthesized rats. The possibility of enterohepatic recirculation was examined using a linked rat technique. Less than 2% of the radioactivity was found in the recipient bile over 9 – 12 h. In eight male rats, the plasma pharmacokinetics of ketoconazole, as determined by an HPLC assay with fluorescence detection, were as follows: Vd = 655 ± 91 ml/kg, Cl = 14.4 ± 5.1 ml/min/kg, and $t_{1/2}$ = 35.0 ± 12.3 min. Three of the rats were given an additional oral dose to determine absolute bioavailability. The time to peak was 30 – 60 min, and the bioavailability was 35.8 ± 3.55%. Previous studies have indicated that ketoconazole is well absorbed in rats; therefore, the poor bioavailability is probably due to first pass metabolism. The prolonged fecal excretion of radioactivity from an intravenous dose was probably caused by slow elimination of ketoconazole metabolites.

Badcock et al (1987) have studied the pharmacokinetics of ketoconazole in seven patients who took it for 1 – 6 months at a dose of 200 mg daily. The mean elimination half-life of the drug was 3.3 h, and although the ketoconazole was given only once daily, a satisfactory clinical response was obtained in all seven individuals. Only a small fraction of the absorbed drug (mean 0.22%) was excreted unchanged in the urine, suggesting almost complete metabolism. The results support the concept that anti-mycotic activity in the tissues continues after the plasma drug concentration has fallen below a critical level. The results also support the concept of a change in pharmacokinetics with chronic dosing. Ketoconazole, an imidazole-piperazine compound, is an orally active antimycotic agent. In addition, ketoconazole is a specific inhibitor of cytochrome P450 3A4. As about 60% of oxidized drugs are biotransformed by this isoform, the potential effect of a concomitant administration of ketoconazole on drug disposition may be of interest during drug development.

One hundred and sixty patients were entered in two multicenter protocols to receive 400 to 2,000 mg of ketoconazole once daily for nonmeningeal or meningeal coccidioidomycosis (Sugar et. al., 1987). For 24

hours after administration of all doses, mean concentrations in serum exceeded MICs for Coccidioides immitis (trough concentrations, > 1 μg/ml). Mean peak concentrations occurred 4 to 6 h after administration, ranging from 7 to 17 μg/ml for doses of 400 to 2,000 mg. Incremental increases in peak concentrations in serum were greatest at doses of 1,200 mg.

To investigate whether long-term therapy altered concentrations in serum, serial data were studied by several methods. The results suggested a trend to increased levels in serum with prolonged therapy, but were not statistically significant. All 168 cerebrospinal fluid (CSF) samples from meningitis patients contained <2.9 μg/ml and only 6 contained > 1 μg/ml. There was no apparent relation between dose, time after dose, site of CSF sampling, or concurrent inflammation and CSF ketoconazole concentration. Neither concentration in serum, toxicity, nor outcome correlated with dose, calculated in milligrams per kilogram at the fixed doses (400-mg increments) under study. Likewise, at the various doses, concentration in serum did not correlate with outcome or toxicity, suggesting that individual drug disposition was not an important factor in outcome or toxicity. Toxicity was reversible, and principal side effects were nausea and vomiting (50%), gynecomastia (21%), decreased libido (13%), alopecia (8%), elevated liver function tests (5%), pruritus (5%), and rash (4%). Gastrointestinal and endocrinologic toxicity were dose related and increased at doses >800 mg. The cumulative percent toxicity requiring discontinuation of drug was 6, 17, 23, and 56% at 400, 800, 1,200, and 1,600 mg doses. Doses of > 400 mg are thus markedly more toxic, and efficacy data for nonmeningeal disease have not demonstrated that they are more efficacious.

Stephen and his coworkers (1991) compared the effects of concomitant sucralfate administration with ranitidine administration on the disposition of a 400 mg ketoconazole. Six healthy male volunteers were randomized to receive 400 mg of ketoconazole alone, 1.0 g of sucralfate concomitantly with a 400 mg ketoconazole dose, or ranitidine, administered 2 hours prior to a 400 mg ketoconazole dose to titrate to a gastric pH of 6. All subjects received all three regimens in crossover fashion. Gastric pH was measured continuously for 4 hours after ketoconazole administration in all

subjects by using a Heidelberg radiotelemetry pH capsule. Relative ketoconazole bioavailability was compared between treatments. With sucralfate, five of six subjects demonstrated a decrease in the peak drug concentration in serum as well as an increase in the time to peak concentration, indicating a delay in ketoconazole absorption. The mean area under the concentration-time curve from 0 to 12 h for ketoconazole following gastric alkalinization was significantly different from that of either ketoconazole alone or ketoconazole with sucralfate ($P < 0.01$). Continuous gastric pH monitoring allowed correlation between the decrease in ketoconazole bioavailability observed with ranitidine and the increase in gastric pH. The apparent decrease in ketoconazole bioavailability observed with sucralfate appears to be caused by an alternative mechanism since a change in gastric pH was not observed. On the basis of these findings, separating the administration of ketoconazole and sucralfate should be considered to decrease the potential for interaction of sucralfate on ketoconazole bioavailability.

The disposition of ketoconazole was characterized in the rat over a wide dose/concentration range (Matthew, 1993). Bolus dose (0.03 – 10 mg/kg) studies indicate that plasma concentration-time profiles for ketoconazole are not superimposable when dose normalized because of nonlinearities occurring in both volume of distribution and clearance. The volume of distribution decreases from 3 to less than 1 L/kg, while the plasma clearance decreases 10-fold from 25 mL/min/kg as the dose is escalated. From these results, infusion rates were calculated to maintain the plasma concentrations achieved with particular bolus doses. The curvilinear relationship between steady-state plasma concentration (0.015 – 8.3 mg/L) and ketoconazole infusion rate (0.021 – 2.45 mg/hr/kg) was analyzed in terms of Michaelis-Menten kinetics. A V_{max} of 3.2 mg/hr/kg and K_m of 2.1 mg/L were obtained by nonlinear regression analysis. At the end of the ketoconazole infusion, liver, adrenals and kidneys were removed and assayed for ketoconazole. Tissue-to-plasma partition coefficients for the liver and adrenals showed a marked dependence upon steady-state plasma concentration. Both parameters (liver, 22; and adrenals, 53) showed a decrease of approximately 10-fold as the plasma concentrations were increased. In contrast, the kidney:

plasma partition coefficient (1.8), blood: plasma concentration ratio (0.6), and plasma binding (96%) of ketoconazole did not show a concentration dependence over the range studied. It is concluded that the liver is an important determinant of ketoconazole's volume of distribution and that saturation of this process accounts largely for the reduction in volume of distribution with increasing dose.

1.16. Aims and Objectives

Aims

To comparison of two dosing regimens of Ketoconazole in patients with Eumycetoma.

Objectives

a) To determine the pharmacokinetic parameters of 400 mg ketoconazole given twice daily.
b) To determine the pharmacokinetic parameters of 800 mg ketoconazole administered twice daily.
c) To compare between the two dosing regimen.

2. Experimental

2.1. Subject and Study site

The study was carried out in the Mycetoma research center, Suba Teaching Hospital, Khartoum Sudan.

Twelve patient adults (males and females) were included in this study. The patients were selected from those who attended Suba hospital out patient clinic and were seen by clinician there. The patients' selection criteria included confirmed eumycetoma caused by Madurella mycetomatis and compliance with follow-up. None of the selected patients take alcohol or smoke cigarettes. No abnormalities were found according to the physical tests and laboratory examinations. The patients were screened to assure that they did not take previous mycetoma treatment prior the trials or any other medication during the trial unless consulting the investigators. Drug that are contraindicated with Ketoconazole, serious concomitant diseases, life expectancy less than 3 month, age less than 18 years are considered as exclusion criteria. Each patient was given a full explanation of the purpose of the study, procedures, duration, discomfort, benefits, confidentiality and the extent to which it is maintained. His/her approval was recorded in a written consent form. The proposal for the study was reviewed and approved by the Ethics Committee of the National Health Laboratory, Khartoum, Sudan.

2.2. Study design and treatment schedule

A comparative open randomized investigation was carried out in two groups of patients. Each group was treated with a different dosing regimen of ketoconazole. Formulations used and treatment schedule are shown in table 2.1.

2.3. Clinical trials

Tow different regimens were used in the study; ten patients were included for each regimen. Only six patient of each regimen completed the study. The two regimens were oral ketoconazole 200 or 400 mg given twice daily (i.e. 400 or 800 mg per day) for three months. After that the response was evaluated and if there were clinical improvement, treatment with ketoconazole was continued for a total of six month. If no improvement had occurred, the patients were treated by the attending physician with a suitable regimen.

Table 2.1. Ketoconazole formulation and doses

Group	Formulation strength	Initial Dose	Subsequent doses (for 6 months)
A	Ketoconazole tablets, 200 mg	2 tablets	1 tablet BD
B	Ketoconazole tablets, 200 mg	4 tablet	2 tablets BD

Each patient took a single dose equivalent to 400 or 800 mg ketoconazole on the first day and the same dose was divided into two doses twelve hours apart on subsequent days for six months. The dose was administered on a full stomach following a standard breakfast, with 250 ml of drinking water. Uniformity of meals was ensured throughout of the trials. No drugs were taken during the trial period. Blood samples (3 ml) were collected at base line 0, and at 1, 2, 3, 4, 6, 8, 12, 16, 20, 24 hours following ketoconazole administration on the first day. Addition blood samples (two samples) were collected on weeks 2, 3, 4 and months 2, 3, 4, 5 and 6. One sample immediately before drugs administration and the other sample at 2 hours after drugs administration, in last month; following the last dose administration, the first day sampling schedule was repeated.

The blood samples were collected in heparinized test tubes and centrifuged at 4000 rpm for 10 minutes and the plasma was separated and transferred by master pipettes to Ependorff tubes and kept in deep freezing at – 20 °C until analysis.

Assessment of patients was carried at entry, weekly for one month, monthly for six months, and at end of the Ketoconazole treatment.

The assessment procedure was as follows:

- Personal data
- Name: .Age: Sex:
- Occupation: Residence: wt:

- Medical history:

- Physical examination:

- Local examination:
  - Skin appearance:
  - Number of discharging sinuses:
  - Degree of disability:
  - Degree of pain:
  - Measurement of lesion circumference:

- Recommended Investigation:
  - Ultrasound:
  - X-ray:
  - Biopsy:
  - Culture:

- Laboratory assessments:
  - Full blood count:
  - Liver enzymes:
  - Serology:

Efficacy parameters:

Skin Colour

- 0 normal color

- 1 abnormal color

Skin attachment:

- 0 normal mobile skin
- 1 mildly fixed to underlying tissue
- 2 severe disability

Number of discharging sinuses number

- Degree of disability
- 1 mild disability
- 2 moderate disability
- 3 severe disability

Measurement of swelling: cm

Radiological finding

- 0 no lesions
- 1 mild bone involvement
- 2 moderate bone involvement
- 3 severe bone involvement

Ultrasound of lesions:

- 0 no grains
- 1 grains present
- 2 tissue reaction

Culture of biopsy

- 0 negative
- 1 positive

Serology:

- 0 negative
- 1 positive
- 1 positive, high intensity

Clinical evaluation

- Complete response cured with relapse
- Partial response < 50 % of sinuses are healed and < 50 % decrease of the swelling
- Stable disease: non of other descriptions
- Progressive disease increasing lesions

Relapse after initial response at day 30 and during follow–up

Evaluation of serology

- No change
- Decrease in number of precipitation lines
- Became negative

Histological Evaluation

- Failure no decrease in number of grain
- Improved decrease number of grain
- Success absence of grain
- Relapse new grains after initial decrease in no

Microbiological Evaluation

- Failure positive culture
- Success negative culture
- Relapse positive culture after earlier success cure

Data Recording

- Data entered on a Patient Record Form.
- Data processed and analyzed.

2.4. Materials and methods

2.4.1. Determination of ketoconazole in plasma

Several analytical procedures have been developed for measurement of ketoconazole concentration in biological fluids. Due to its high selectivity, good sensitivity and reproducibility, the HPLC method was used by several researchers as the method of choice for the determination of ketoconazole in biological fluids of both man and animals. The first HPLC method for the determination of ketoconazole in plasma was described by Alton (1980); this method involved lengthy multiple extractions and did not employ internal standards. Subsequently, Pascucci et al. (1983) described a simplified sample preparation technique employing C_{18} reversed- phase cartridges and phenothiazine as an internal standard. Riley and James (1986) found direct procedure to be applicable to the analysis of ketoconazole in the lung, liver and adrenal of the rat and alternative strategies are presented in their study. Turner et al. (1986); Yuen and Peh (1998); Ramos et al. (2000); Khashaba et al. (2000), Bruijn et al. (2001), Farhadi and Maleki, (2001) and Chen et al. (2002), all used the HPLC technique for the determination of ketoconazole levels in different studies. Therefore in present study the plasma concentrations of ketoconazole were measured by HPLC.

2.4.2. Materials

All solvents were HPLC grade and the chemicals were analytical grade. Ketoconazole was a gift from Pharmaline Pharmaceutical (Lebanon).

2.4.3 Instrumentation and chromatographic system

A high performance liquid chromatographic (HPLC) method for the determination of ketoconazole in human plasma using fluorescence detector is described.

The drug was extracted from 200 µl of plasma with 7 ml t-butylmethylether. The extract was evaporated at 45°C and the residue was reconstituted with 300 µl of mobile phase and injected into the HPLC system (20 µl).

The drug and the internal standard (Itraconazole) were eluted from Symmetry C_8 column (3.9*150 mm, 5µm) at 30 °C with a mobile phase consisting of (de-ionized water-acetonitrile-methanol (40:50:20% v/v/v) and 1ml triethylamine, then adjusted to pH 7.0 using 30% glacial acetic acid, at flow rate of 2.0 ml/min. The separation was monitored using a fluorescence detector at λex 250 nm and λem 370 nm.

2.5. Data analysis

Quantitation was achieved by measurement of the peak area ratio of the drug to the internal standard following validation of the HPLC procedure. The peak area ratios of drug/internal standard were plotted against the concentrations. The slope, intercept and correlation coefficient were determined by weighted ($1/\chi$) linear least squares regression.

The analysis is based on measuring the concentration of ketoconazole in plasma. The area under the plasma concentration-time curve between 0 and 24 hr ($AUC_{0\rightarrow24}$) was calculated by trapezoidal rule and the AUC to infinity ($AUC_{0\rightarrow\infty}$) was estimated from the slope of the declined phase and the last measured concentration. The maximum drug concentrations in the plasma (C_{max}) and the time when it was reached (T_{max}) were obtained directly from plasma concentration versus time data. The $AUC_{0\rightarrow\infty}$ of ketoconazole together with C_{max} and T_{max} were used to describe the extent and rate of its bioavailability, respectively.

A non-compartmental model using simple Excel® Software package was employed for calculation of pharmacokinetic parameters. The elimination rate constant (Ke) and half- life of elimination ($T_{½}$) were calculated by linear regression of terminal slope of excretion rate profiles. The $AUC_{0\rightarrow24}$ hr was calculated by trapezoidal rule and the $AUC_{0\rightarrow\infty}$ was obtained by adding the C_{final}/ke to $AUC_{0\rightarrow24}$ hr, where the C_{final} is the last measurable concentration. The mean residence time ($MRT_{0\rightarrow\infty}$) was obtained by calculating the ratio of the area under the first moment curve for 0 to infinity ($AUMC_{0\rightarrow\infty}$) by $AUC_{0\rightarrow\infty}$.

2.6. Statistical analysis

The computer program Excel was used for statistical analysis. All parameters were expressed as (Mean ± SD). The coefficient of variation (C.V) was calculated for the pharmacokinetic parameters.

3. Results

3.1. Analytical procedure

A high performance liquid chromatographic method (HPLC) for the determination of ketoconazole in human plasma using fluorescence detection was used. The HPLC analytical method exhibited good reproducibility and selectivity. It permitted simultaneous determination of ketoconazole and itraconazole (internal standard) in small amounts in plasma with no interference from metabolites or endogenous materials. Figures 3.1 – 3.6 show representative chromatograms for blank and internal standard plus zero, 0.20, 0.50, 11.00, 16.00 μg/ml of ketoconazole in plasma.

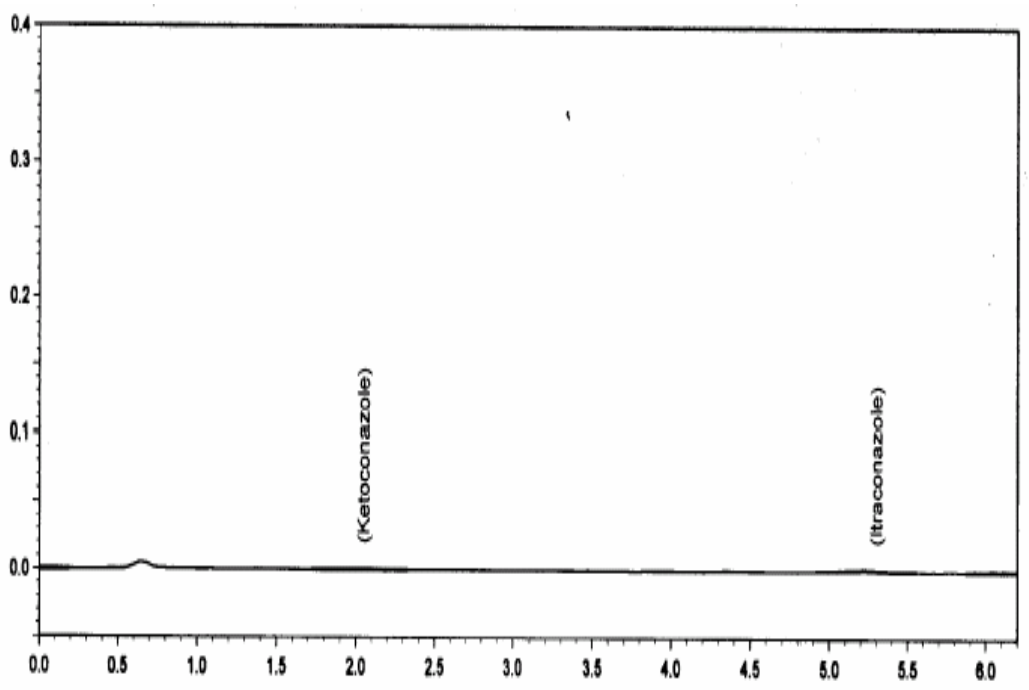

Figure 3.1. Chromatogram of Blank plasma

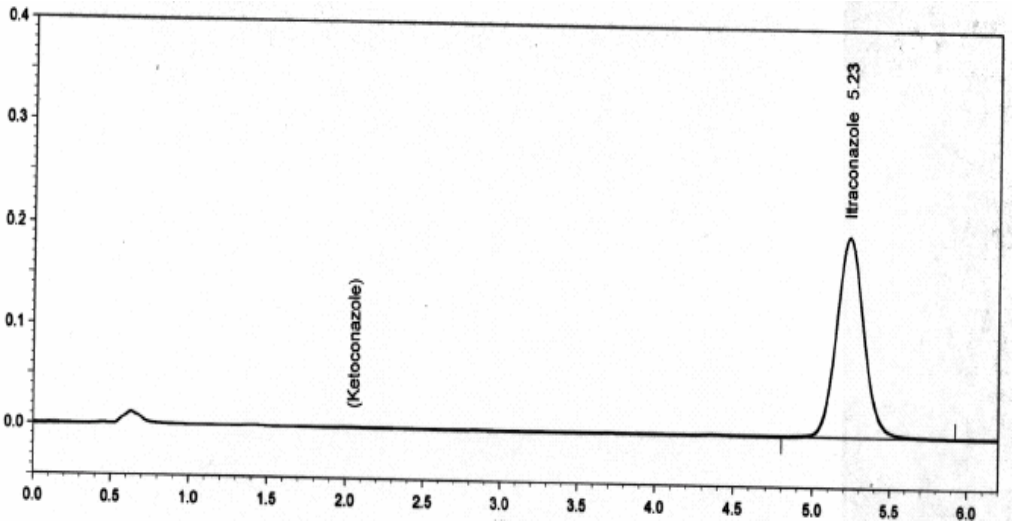

Figure 3.2. Chromatogram of blank plasma spiked with itraconazole as internal standard

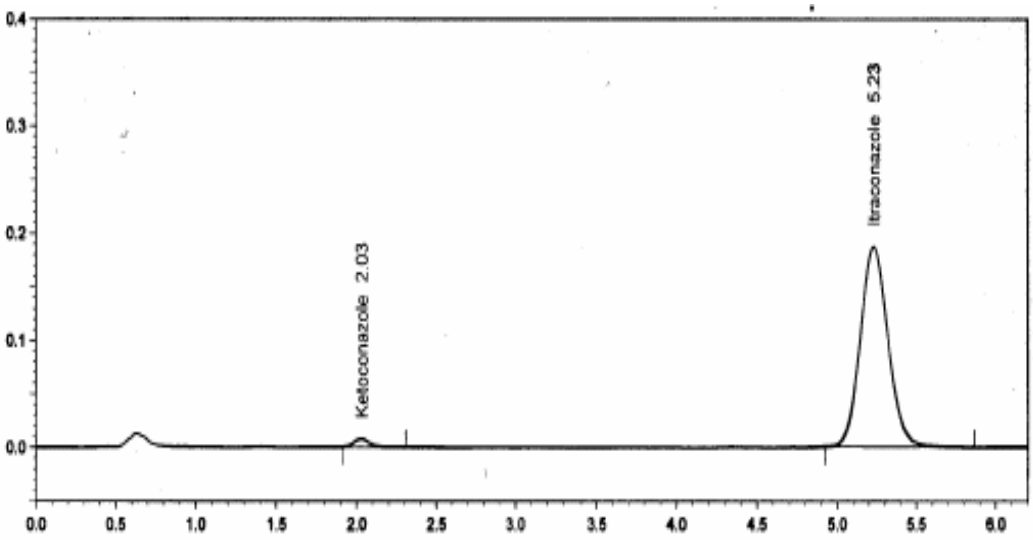

Figure 3.3. Chromatogram of plasma spiked with 0.2 μg/ml of ketoconazole (Rt = 2.03 min) and itraconazole (Rt = 5.23 min)

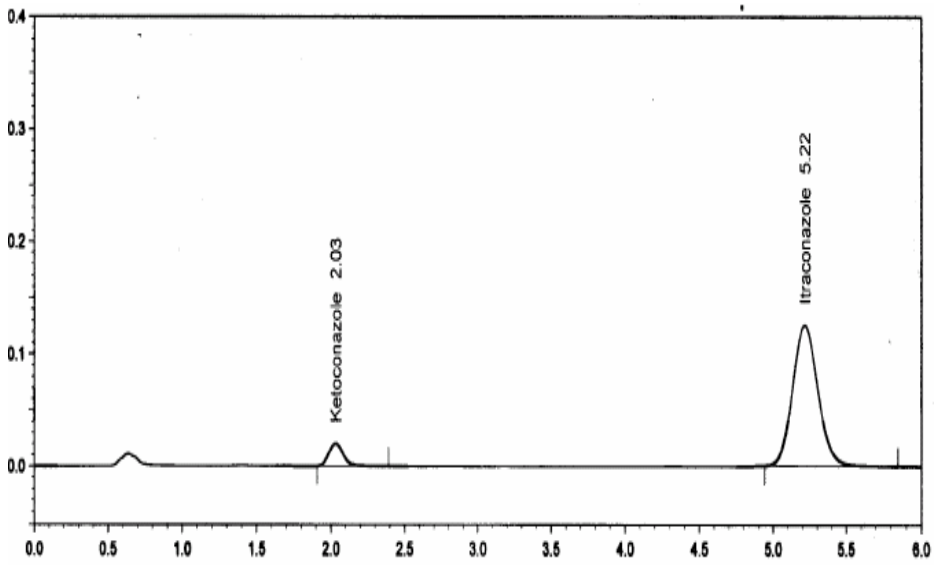

Figure 3.4. Chromatogram of plasma spiked with 0.5 μg/ml of ketoconazole (Rt = 2.03 min) and itraconazole (Rt = 5.23 min)

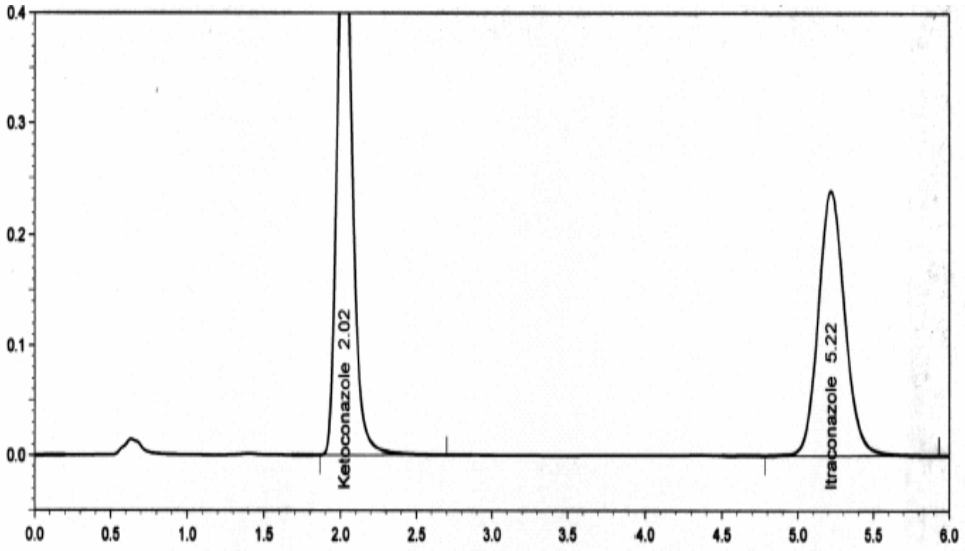

Figure 3.5. Chromatogram of plasma spiked with 11 μg/ml of ketoconazole (Rt = 2.03 min) and itraconazole (Rt = 5.23 min)

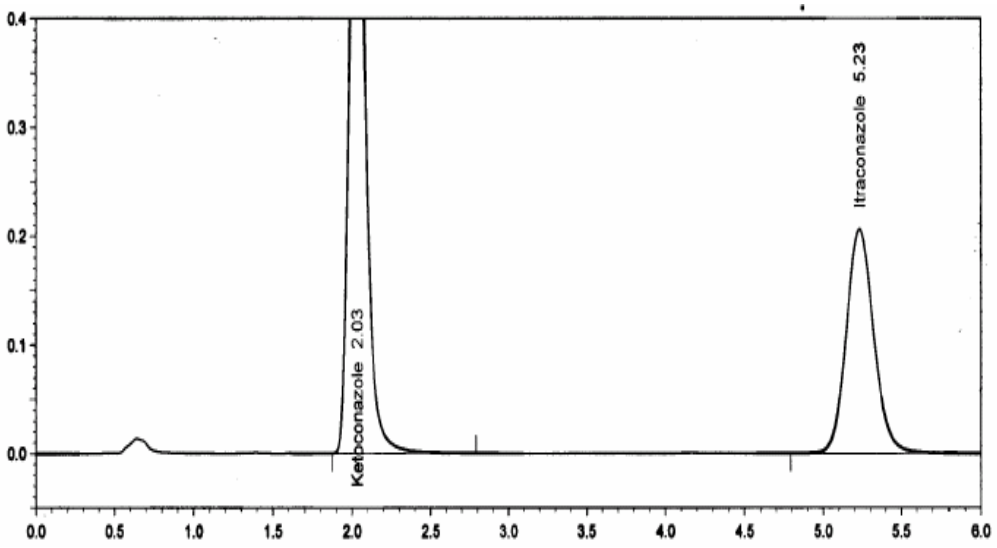

Figure 3.6. Chromatogram of plasma spiked with 16 μg/ml of ketoconazole (Rt = 2.03 min) and itraconazole (Rt = 5.23 min)

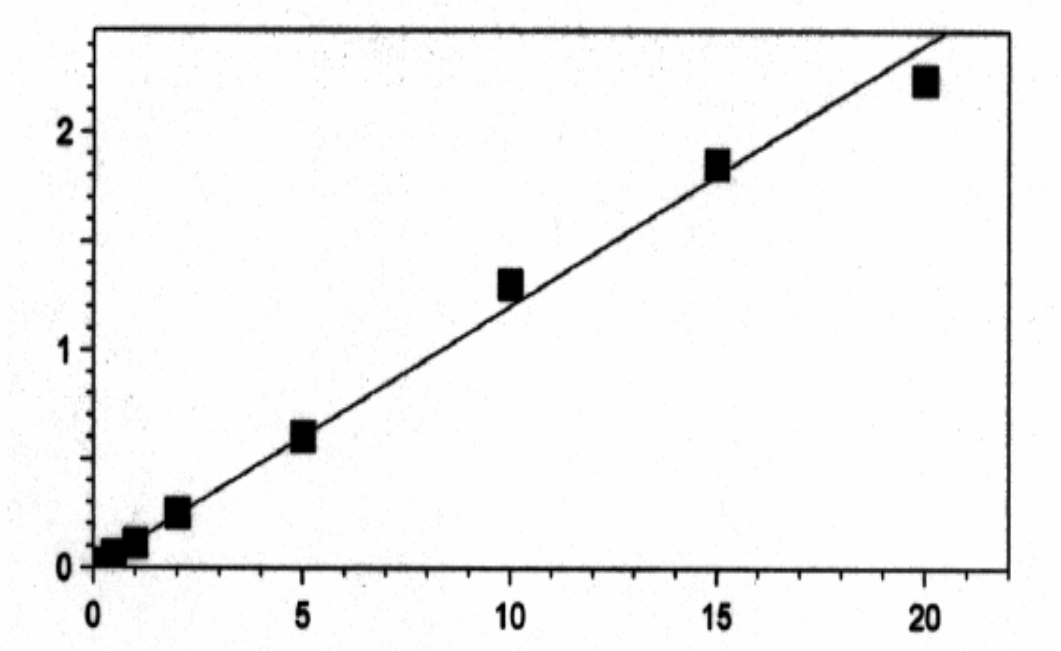

Figure 3.7. Calibration curve of ketoconazole in plasma (r =0.992705)

Quantitation was achieved by measurement of the peak area ratio of the drug to the internal standard. The linearity for ketoconazole in plasma in a range of concentration of 0.20-20 μg/ml and through a range of concentration 50-6000 ng/ml was validated. Standard curves of ketoconazole were analyzed at concentrations 0.20, 0.50, 1.00, 2.00, 5.00, 10.00, 15.00, and 20.00 μg/ml.

Good linearity was achieved (R2 = 0.992705). Calibration curve of ketoconazole in plasma is illustrated in figure 3.7.

3.2. Pharmacokinetics of ketoconazole tablets; 400 mg regimen

Tables 3.1 illustrates individual plasma concentration versus time profiles of ketoconazole after its oral administration to 6 patients. The plasma concentrations of ketoconazole after the first dose (single, 400 mg) are represented by samples number 1 to 11. Samples number 12 to 27 represent plasma concentration following the administration of repeated doses of ketoconazole (up to six months, 200 mg twice a day). Samples 28 to 38 correspond to plasma concentrations following the administration of the last dose (single, 400 mg) of ketoconazole tablets. The table shows the individual variation between the subjects. Most of the patients finished the first three months of therapy and stopped, except for two. One patient did not complete the full course of the trial and dropped out following the first week and the other patient completed the whole six months of the trial. Therefore, data following the administration of the last dose is represented by only one patient.

Table 3.1: Ketoconazole Plasma concentration (μg/ml) versus time (h) following oral administration of 400 mg regimen for 6 patients.

Sample No.	Time (h)	Day	Weeks	BH	NO	EM	FO	RH	SY	Mean	SD	C V%
1	0	1	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0
2	1			1.19	7.95	1.38	6.31	2.52	9.35	4.78	3.54	74.11
3	2			5.69		2.82	8.50	10.32	9.65	7.40	3.11	42.06
4	3			15.07	8.09	2.73		6.26	6.16	7.66	4.57	59.67
5	4			12.21	7.38	1.17	4.26	5.29	4.85	5.86	3.70	63.18
6	6			8.70	4.26	0.37	4.31	3.42	2.96	4.00	2.71	67.81
7	8			8.21	2.80	0.21	2.86	2.84	2.28	3.20	2.66	83.02
8	12			3.86	1.25		1.29	1.17	1.70	1.85	1.14	61.49
9	16			1.40	0.66		1.10	0.38	1.00	0.91	0.40	43.63
10	20			1.25	0.63	0.61	0.93			0.86	0.30	35.24
11	24				0.70	0.50	0.47			0.56	0.13	22.46
12	192	7	1		6.26	0.71	2.75	1.40	0.82	2.39	2.31	96.80
13	194			4.27	14.03	3.63	8.96	1.56	4.33	6.13	4.57	74.49
14	360	14	2		1.54	0.95	0.25		0.59	0.83	0.55	66.25
15	362				14.91	5.91	4.74		5.32	7.72	4.82	62.40
16	528	21	3		4.27		1.22			2.75	2.16	78.57
17	530				12.79		5.41			9.10	5.22	57.35
18	1368	56	8	5.12	3.78		1.29		0.49	2.67	2.15	80.59
19	1370			6.75	5.32	4.75	10.18		7.22	6.84	2.12	30.98
20	2040	84	12	2.46	0.28	1.62	6.05			2.60	2.47	94.81
21	2042			7.22	13.24	5.53			14.78	10.19	4.51	44.21
22	2904	119	17		0.72		6.06		7.47	4.75	3.56	74.96
23	2906				13.81					13.81		0.00
24	3552	147	21		5.82				2.76	4.29	2.16	50.44
25	3554				12.81				9.99	11.40	1.99	17.49
26												
27												
28	4224	0	25		3.34							
29	4225	1			3.67							
30	4226	2			5.27							
31	4227	3			5.92							
32	4228	4			9.49							
33	4230	6			11.56							
34	4232	8			9.92							
35	4236	12			6.62							
36	4240	16			5.04							
37	4244	20			4.34							
38	4248	24			2.46							

Plasma concentrations versus time profiles following oral administration of 400 mg ketoconazole to 6 patients were illustrated in figures 3.8, 3.9, 3.10, 3.11, 3.12, and 3.13.

A total of fifteen samples were collected from patient BH in 3 month as shown in figure 3.8. The highest concentration achieved following the first dose was 15.07 µg/ml in 3 hrs, the levels declined to 1.25 µg/ml in 20 hrs and fluctuated between 6.75, 2.46 and 7.22 µg/ml following the administration of the repeated dose for 2 month. It is worth mentioning that this patient showed the highest levels among all volunteers.

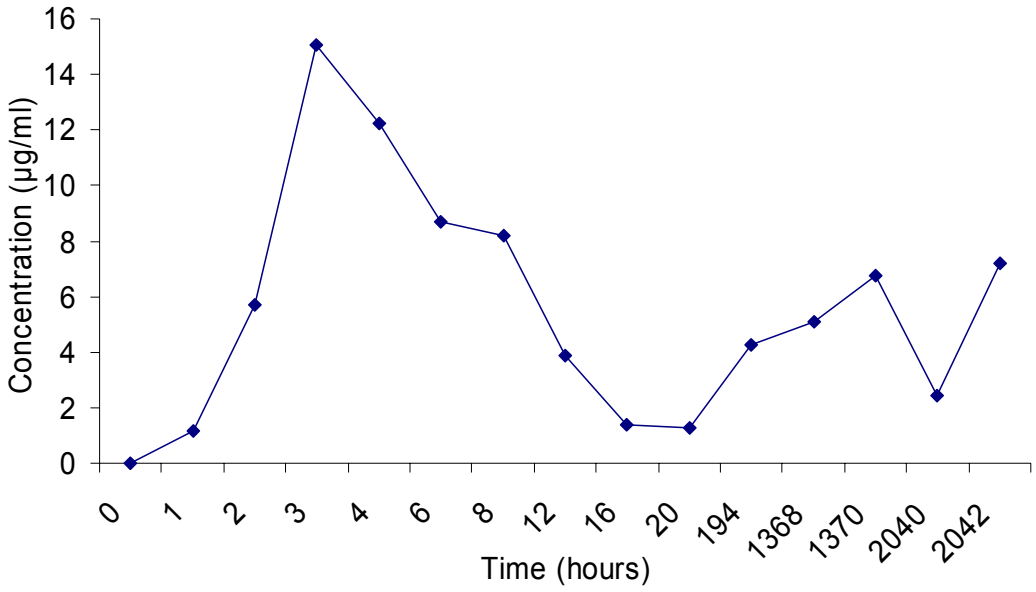

Figure 3.8: Plasma concentration-time profile following the administration of 400 mg ketoconazole regimen to patient BH

Thirty five samples were collected from patient NO during 6 months. These characterize the full profile as described in the study protocol. The plasma concentration – time profile was plotted in figure 3.9. The concentration increased until it reached a maximum of 8.09 µg/ml in 3 hrs in the first day then declined to 0.7 µg/ml in 24 hrs. At steady state plasma levels fluctuated between trough and peak concentrations for six months. The profile shows a maximum of 11.56 µg/ml in 6 hrs following the administration of the last dose and a decrease to 2.46 µg/ml in 24 hrs.

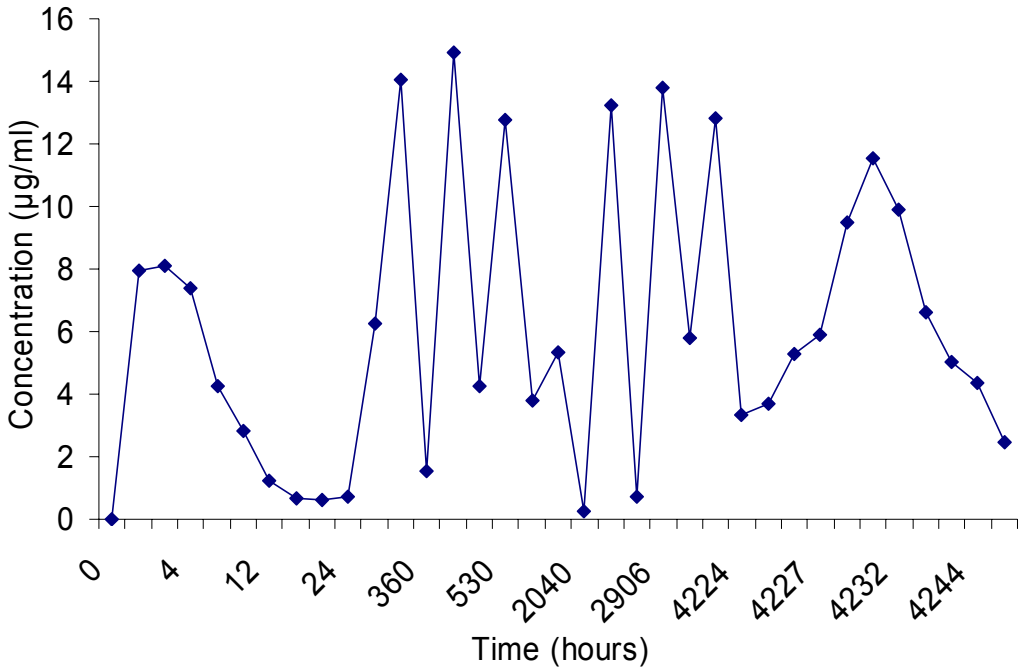

Figure 3.9: Plasma concentration-time profile following the administration of 400 mg ketoconazole regimen to patient NO

Few samples were analyzed for patient EM; about sixteen samples which are represented in figure 3.10. The levels of this patient represent the lowest concentrations compared to those of the other 6 patients. His concentration increased and reached a maximum of only 2.82 µg/ml in 2 hrs and then gradually declined to 0.5 µg/ml in 24 hrs. The levels increased to a maximum concentration and decreased to a minimum concentration during the next 12 weeks.

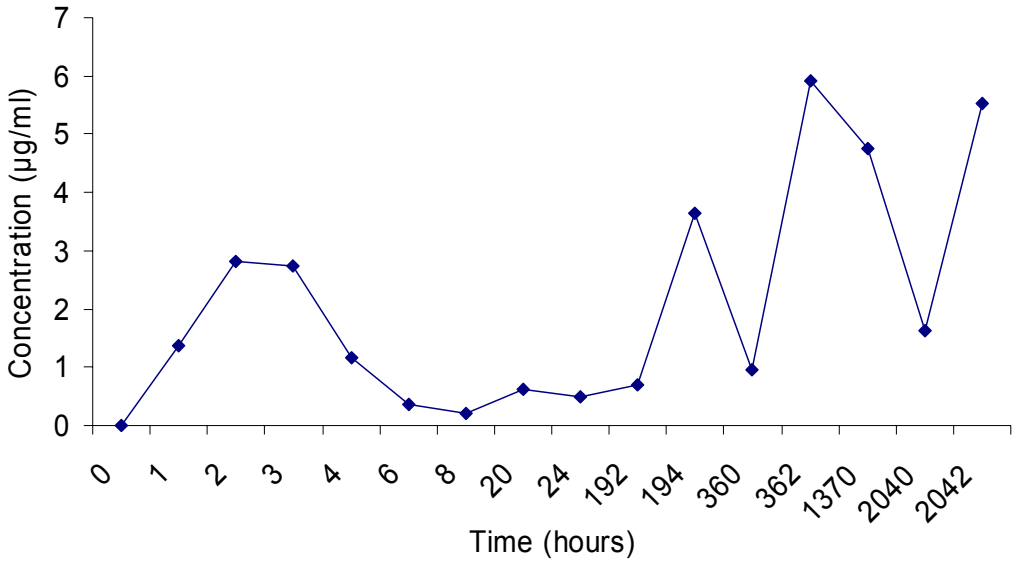

Figure 3.10: Plasma concentration-time profile following the administration of 400 mg ketoconazole regimen to patient EM

Figure 3.11 depicts the plasma concentration against time profile for patient FO. This patient complied with the sampling schedule till the 17th week where the last two peak samples were observed to be missing. The profile shows increase to a maximum concentration of 8.5 µg/ml in 2 hrs following the administration of the first dose and a decline to 0.47 µg/ml in 24 hrs. Fluctuation between maximum and minimum concentrations at steady state was presented for multiple dose administration till week 17.

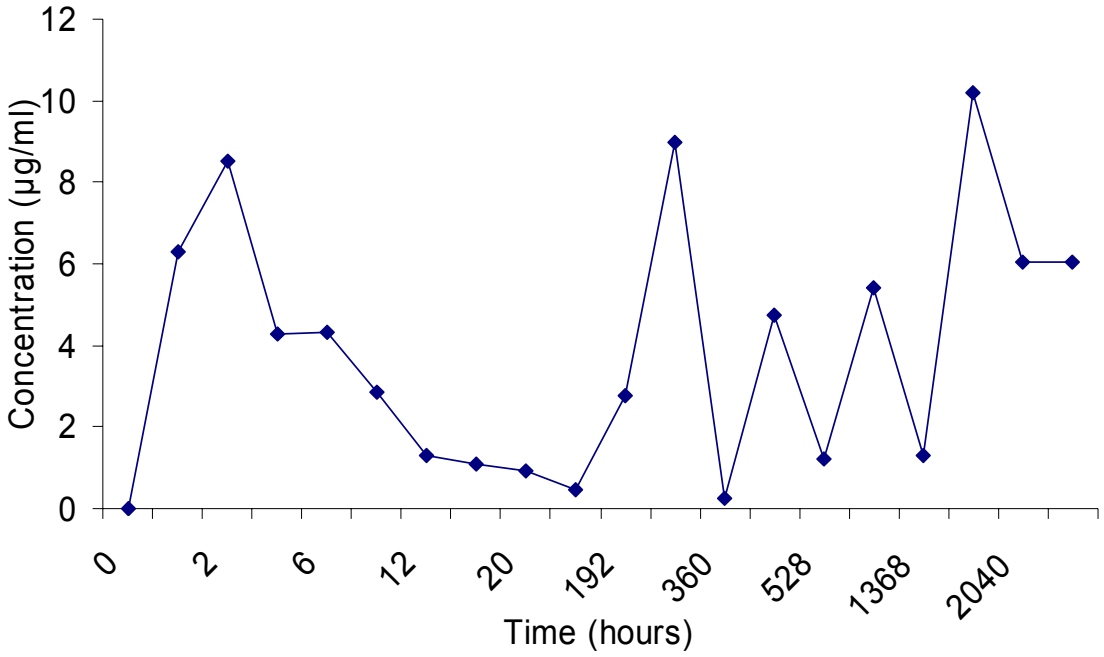

Figure 3.11: Plasma concentration-time profile following the administration of 400 mg ketoconazole regimen to patient FO

Eleven samples only were collected from patient RH. This patient dropped from the study after the first week. Plasma levels are illustrated in figure 3.12 where the concentration increased after 2 hrs to 10.32 µg/ml and decreased to 0.38 µg/ml in 16 hours. Only one peak and one trough samples were collected.

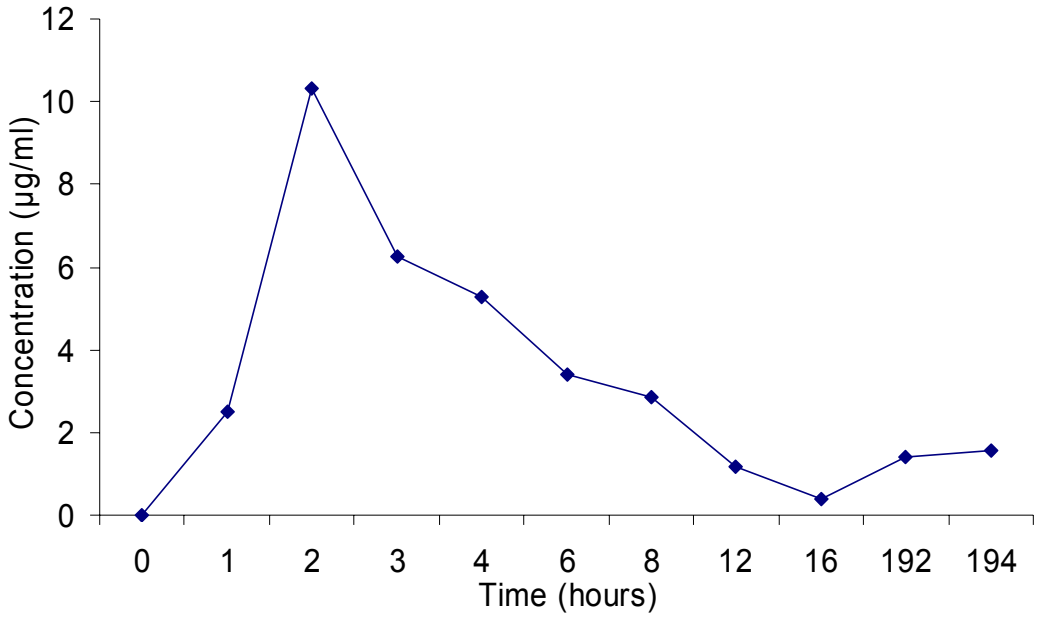

Figure 3.12: Plasma concentration-time profile following the administration of 400 mg ketoconazole regimen to patient RH

Patient SY provided twenty one samples, the levels of these samples increased and reached a maximum of 9.65 µg/ml after 2 hrs then decreased gradually until 1.0 µg/ml in the first day. At the first week plasma concentrations measured immediately before the dose and 2 hours after administration of the dose were found to be 0.82 and 4.33 µg/ml, respectively. The levels fluctuated between trough and peak concentrations for five months. The last trough and peak levels reported were 0.27 and 5.37 µg/ml, respectively. The highest concentration achieved by this patient was 14.78 µg/ml following 3 moths of therapy (figure 3.13).

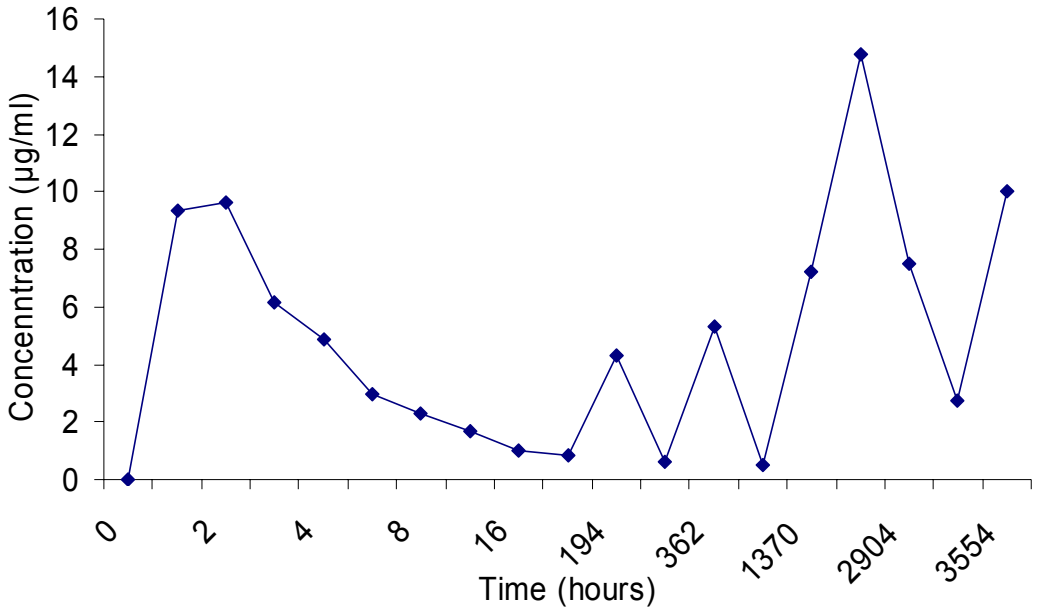

Figure 3.13: Plasma concentration-time profile following the administration of 400 mg ketoconazole regimen to patient SY

Figure 3.14 represents the plasma concentration – time profiles for the 6 patients after oral administration of ketoconazole 400 mg regimen. As can be seen in the figure, most patients finished three months of the study. On the other hand, one patient (RH) finished only the first week of sampling while patient SY stopped after five months. Only one patient (NO) was able to complete the full course of the trail. From sample number 28 the graph shows the plasma concentrations for this patient. Interindividual variability was observed between the patients.

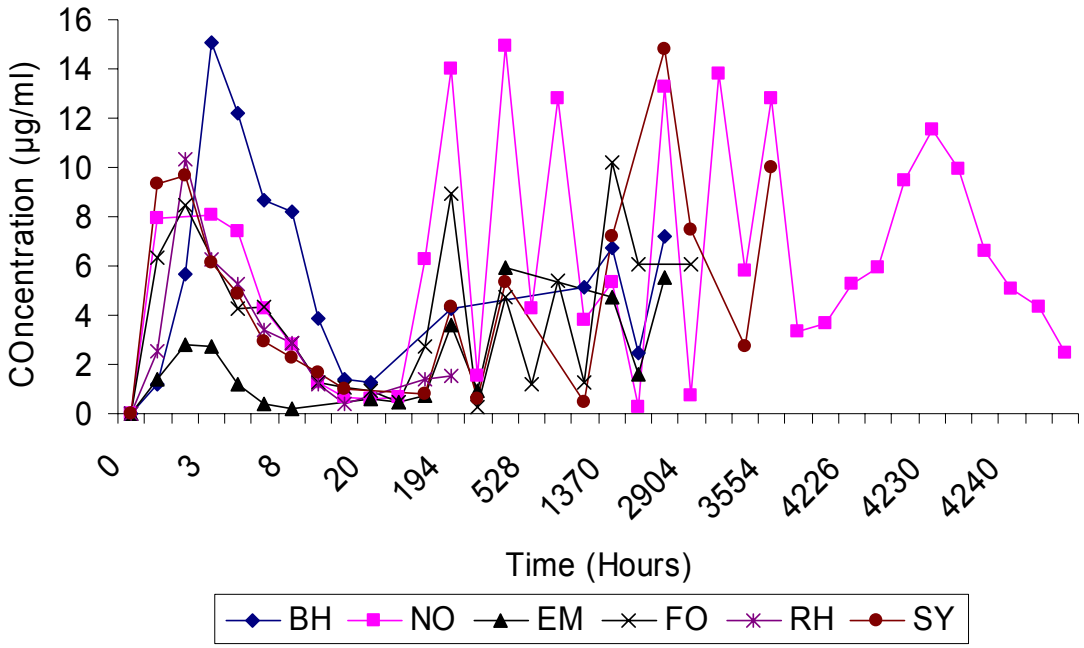

Figure 3.14: Plasma concentration-time profile following the administration of 400 mg ketoconazole regimen to 6 mycetoma patient

Mean (± SE) plasma concentration versus time profiles of ketoconazole tablets following the administration of 400 mg regimen to 6 patients is depicted in figure 3.15. No error bars were included in the last part of the profile since the data is collected from only on patient.

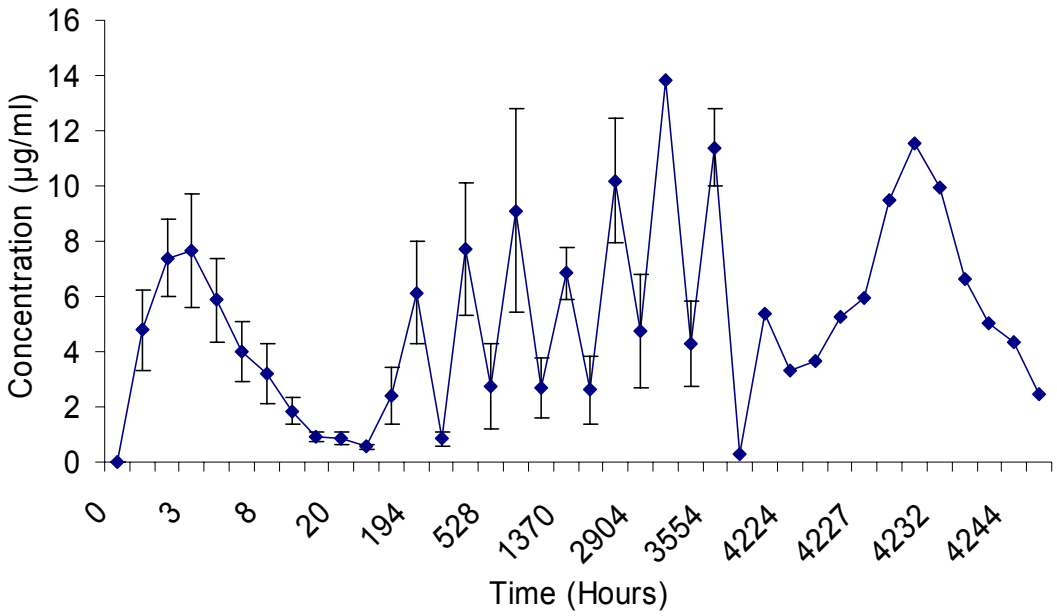

Figure 3.15: Mean (± SE) plasma concentration-time profile following the administration of ketoconazole 400 mg regimen to 6 mycetoma patients

Figures 3.16 and 3.17 represent individual plasma concentration against time profiles of the six patients and the mean (± SE) plasma concentration of ketoconazole tablets following the administration of the first dose of 400 mg regimen, respectively. The profiles for the majority of the patients were similar except for two patients. One patient (EM) showed the lowest plasma levels while patient BH showed the highest levels following the administration of the first dose.

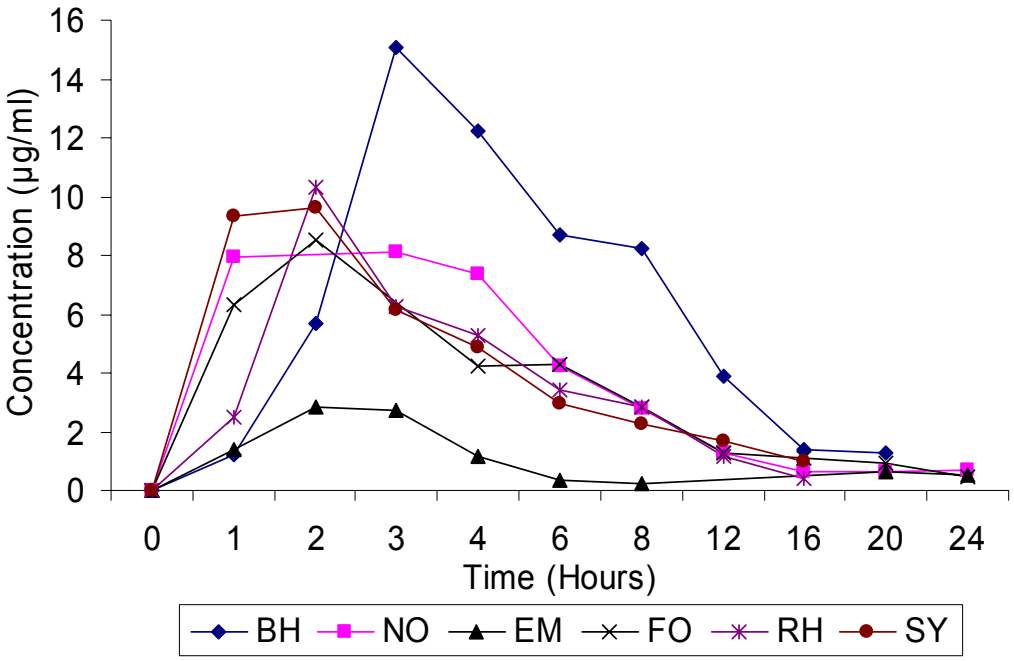

Figure 3.16: Plasma concentration-time profile following administration of the first dose of 400 mg ketoconazole regimen to 6 mycetoma patients

After plotting Mean (± SE) plasma concentration versus time following the administration of first dose of 400 mg ketoconazole regimen in figure 3.17, the concentration started to increase from 0 until it reached a maximum concentration of 7.66 µg/ml at 3 hrs and then decline.

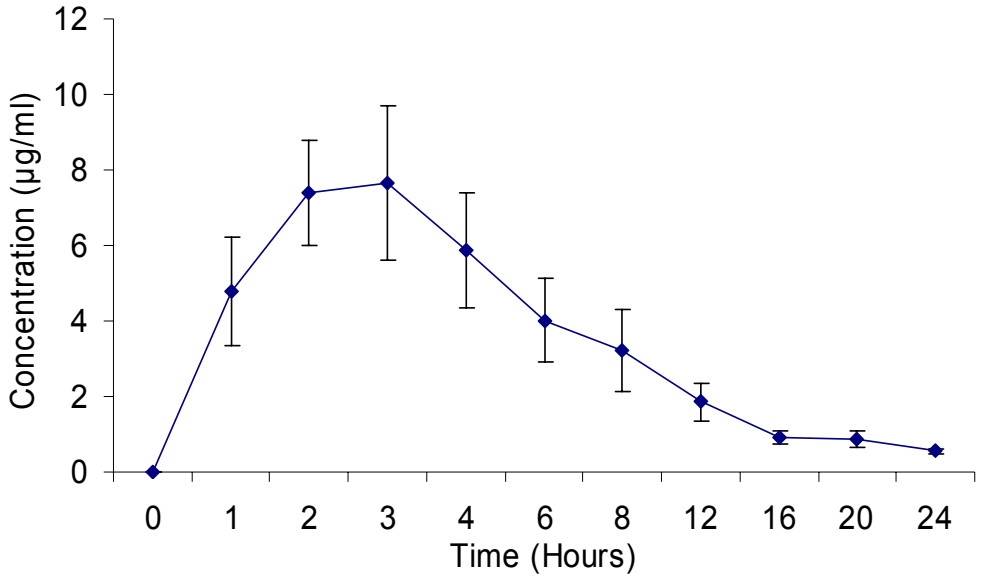

Figure 3.17: Mean (± SE) plasma concentration-time profile following the administration of the first dose 400 mg ketoconazole regimen to mycetoma patients (n = 6)

Figures 3.18 and 3.19 represent the plasma concentration versus time profiles of first and last dose for 400 mg regimen for patient NO.

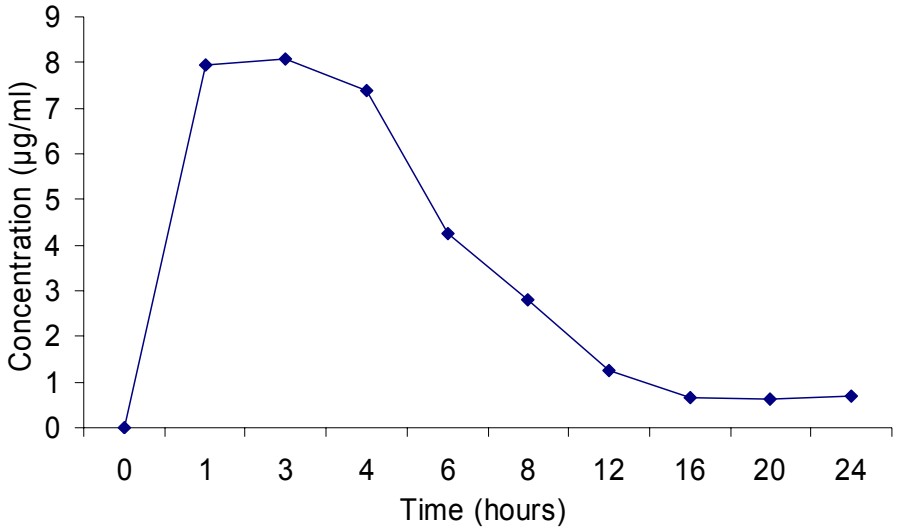

Figure 3.18: Plasma concentration-time profile following administration of the first dose of 400 mg ketoconazole regimen to patient NO

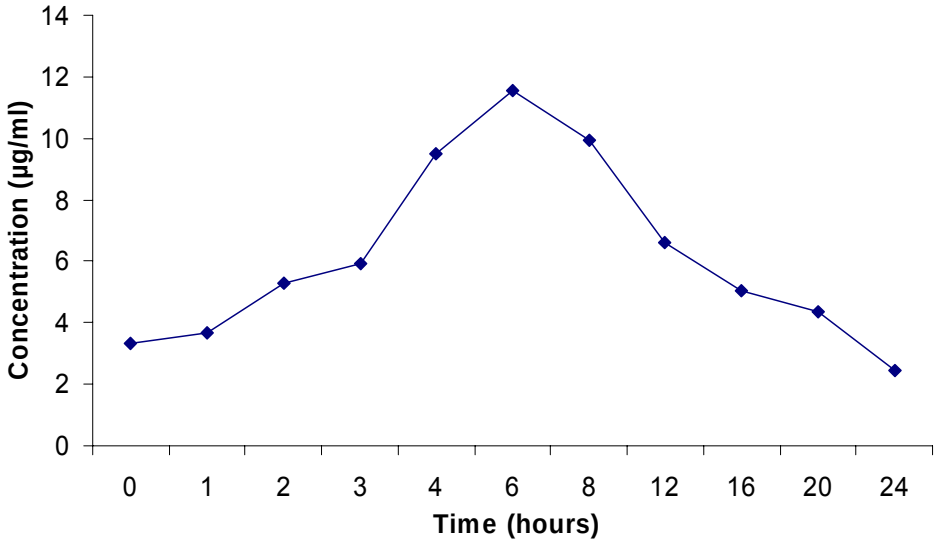

Figure 3.19: Plasma concentration-time profile following administration of the last dose of 400 mg ketoconazole regimen to patient NO

Comparison of first and last doses of 400 mg regimen for patient NO, the only one who completed the trial, is shown in figure 3.20. In the first dose profile the concentration started to increase from 0 to a maximum concentration of 8.09 µg/ml at 3 hrs and then decreased. While in the last dose profile the concentration started from 3.34 µg/ml achieved highest concentration of 11.56 µg/ml at 6 hrs then declined gradually as exposed in figure 3.20 to 2.46 µg/ml. it has been observed that there is a shift of the profile upward and to the right.

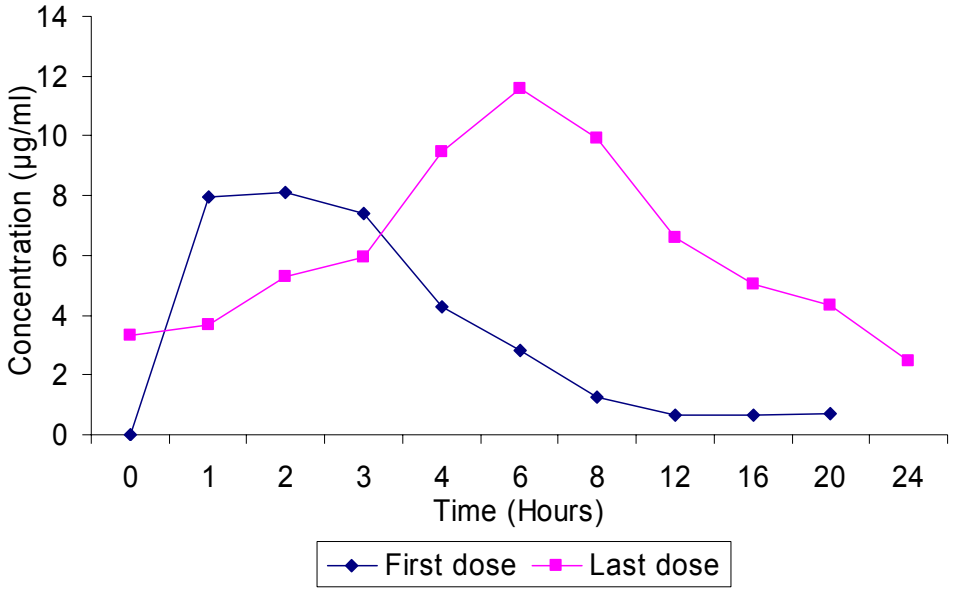

Figure 3.19: Plasma concentration-time profile following administration of the first and last dose of 400 mg ketoconazole regimen to patient NO

Table 3.2. ketoconazole pharmacokinetic parameters after oral administrations of the first dose of 400 mg regimen to 6 eumycetoma patients.

Patient	Parameter									
	T_{max} (h)	Cp_{max} (µg/ml)	AUC∞ (µg.h/ ml)	AUMC (µg.h^2/ ml)	MRT (h)	K_e (h^{-1})	$t_{1/2}$ (h)	CL/F (L/h)	Vd/F (L)	AUC24 (µg.h/ml)
BH	3.00	15.07	113.83	968.37	8.51	0.16	4.43	3.51	22.47	
NO	3.00	8.09	67.13	499.27	7.44	0.20	3.48	5.96	29.94	63.61
EM	2.00	2.82	17.82	187.31	10.51	0.48	1.45	22.45	46.94	16.78
FO	2.00	8.50	63.77	578.55	9.07	0.10	7.04	6.27	63.70	59.00
RH	2.00	10.32	49.91	295.81	5.93	0.18	3.79	8.01	43.82	
SY	2.00	9.65	61.56	459.41	7.46	0.13	5.27	6.49	49.35	
Mean	2.33	9.08	62.34	498.12	8.15	0.21	4.24	8.78	42.70	46.46
SD	0.52	3.96	31.03	270.79	1.58	0.14	1.87	6.85	14.67	25.81
C V%	22.32	43.61	49.78	54.36	19.39	66.67	44.10	78.02	34.36	55.55

Table 3.3: Ketoconazole pharmacokinetic parameters after oral administrations of the last dose of 400 mg regimen to patient NO.

Patient	Parameter									
	T_{max} (h)	Cp_{max} (µg/ml)	AUC∞ (µg.h/ ml)	AUMC (µg.h^2/ ml)	MRT (h)	K_e (h^{-1})	$t_{1/2}$ (h)	CL/F (L/h)	Vd/F (L)	AUC24 (µg.h/ml)
NO	6.00	11.56	183.07	2681.67	14.65	0.08	8.60	1.09	13.55	152.57

Tables 3.2 and 3.3 represent the pharmacokinetic parameters of 400 mg following the first and last doses, respectively achieved after oral administration of ketoconazole tablets.

The mean (± SD) values of maximum drug concentration (Cp_{max}) after oral administration of the first and last doses of 400 mg regimen are in the order of 9.08 ± 3.96 µg/ml (range, 2.82 – 15.07 µg/ml) and 11.56 µg/ml (values generated from data of only one patient). coefficient of variation (CV%) following the administration of the first dose was 43.59%. On the other hand, the mean values of times to reach Cp_{max} (T_{max}) were found to be 2.33 ± 0.52 hr (range, 2 – 3 hrs, CV% = 22.13%) following first dose and 6 h after last dose.

The mean (± SD) values of AUC_{∞} and $AUMC_{\infty}$ for ketoconazole obtained after oral administration of first dose of 400 mg regimen were 62.34 ± 31.03 µg.h/ml (range, 17.82 – 113.83 µg.h/ml) and 498.12 ± 270.79 $\mu g.h^2/ml$ (range, 187.31 – 968.37 $\mu g.h^2/ml$), respectively. Values obtained after oral administration of last dose were 183.07 µg.h/ml and 2681.67 $\mu g.h^2/ml$. CV% following the first dose were estimated as 49.77% and 54.36% for AUC_{∞} and $AUMC_{\infty}$, respectively. Comparing the AUCs of three patients, the percentage AUC_{24} in relation to the AUC_{∞} was 94%. This indicates that the estimated areas were less than 10% of the total area. This is a proof of the accuracy of the sampling schedule.

The mean (± SD) values of MRT were determined as 8.15 ± 1.58 hr (range, 5.93 – 10.51 hrs) and 14.65 hr following ingestion of first and last doses, respectively.

CL/F values (mean ± SD) for 400 mg ketoconazole regimen were found to be 8.78 ± 6.85 L/h (range, 3.51 - 22.45 L/h, CV% = 77.98%) and 1.09 L/h for first dose and last doses, respectively.

Vd/F values (mean ± SD) were found to be in the sequence of 42.70 ± 14.67 L (range, 22.47 – 63.70 L and CV% 34.35%) and 13.55 L for first dose and last doses of 400 mg ketoconazole regimen.

The mean (± SD) values of the elimination half - life ($T_{1/2}$) for ketoconazole are calculated as 4.24 ± 1.87 hr (range, 1.45 – 7.04 hrs) and 8.60 hr following the first and last doses of the 400 mg regimen, respectively. Coefficient of variation for the values of half – life was 44.07%.

3.3. Pharmacokinetics of ketoconazole tablets; 800 mg regimen

Table 3.4 represent the individual plasma concentrations of all doses, first dose and last dose, respectively after oral administration ketoconazole tablets 800 mg regimen to 6 patients. Individual variations between the patients are observed.

Tables 3.4 represents individual plasma concentration-time profiles of ketoconazole after oral administration of the 800 mg regimen to 6 eumycetoma patients. The sampling schedule was the same as that of the 400 mg regimen. The plasma concentrations after the first dose (single, 800 mg) are represented by samples number 1 to 11. Samples number 12 to 27 represent plasma concentration following multiple dose administration of ketoconazole (up to six months, 400 mg twice a day). Samples 28 to 38 correspond to plasma concentrations following the administration of the last dose (single, 800 mg) of ketoconazole tablets. Unlike the first regimen, patient compliance was quite reasonable. Most of the patients finished six months of therapy except for two patients (SE and OE) who stopped after two weeks of sampling. Interindividual variations between the subjects were noted to the same extent as those in the 400 mg regimen (variable CV% values).

Table 3.4: Ketoconazole Plasma concentration (μg/ml) versus time (h) following oral administration of 800 mg regimen for 6 patients.

Sample No.	Time (h)	Day	Weeks	LAA	SH	AAO	SE	AAM	OE	Mean	SD	CV%
1	0	1	0	0	0	0	0	0	0	0.00	0.00	0
2	1			6.01	11.2	10	17.1	4.3	9.1	9.61	4.47	46.5
3	2			10.2	14.7	16	30.4	9.4	21	16.92	7.80	46.1
4	3			11.9	11.1	14	27	3.7	18	14.41	7.85	54.5
5	4			5.75	8.15	11	21.2	2.9		9.83	7.04	71.6
6	6			5.16	3.32	5		2.9	8.8	5.03	2.32	46.1
7	8				3.72				7	5.37	2.33	43.4
8	12			2.41	2.24	2.5	10.4	1.2	4.1	3.79	3.37	89.0
9	16			1.07	1.24	0.7	2.59	0.3	1.8	1.29	0.80	62.3
10	20				1.11		1.54		1	1.21	0.30	24.6
11	24				0.88		0.56		0.4	0.62	0.24	38.9
12	192	7	1		1.01		6.81		9.1	5.62	4.15	73.8
13	194				9.72	15	11.1	7	19	12.28	4.54	37.0
14	360	14	2			8.7	5.74	0.6	3.2	4.57	3.48	76.1
15	362				9.01	15	13.7	6.4	21	12.97	5.57	42.9
16	528	21	3	8.67		4.9		0.8		4.78	3.96	82.8
17	530			12.8	12.8	13		1.9		10.21	5.56	54.5
18	1368	56	8	4.8	8.42	0.3		1.1		3.66	3.73	102.0
19	1370			14.7	13.8	16		3.9		12.09	5.57	46.1
20	2040	84	12		7.76	0.4		0.9		3.01	4.12	136.7
21	2042			9.55	8.86	8.7		3.6		7.66	2.75	35.9
22	2904	119	17			7.6				7.55		0.0
23	2906			7.58		11		4.2		7.52	3.31	43.9
24	3552	147	21	5.18		6		3.4		4.86	1.36	28.0
25	3554			14.5		13		3.5		10.40	6.00	57.7
26						5.3		1.6		3.44	2.65	77.2
27						15		12		13.62	1.84	13.5
28	4224	0	25	4.62	0.72			0.5		1.95	2.31	118.3
29	4225	1		4.9	7.8	8.8		4.4		6.48	2.18	33.7
30	4226	2		15.6	18.4	18		11		15.75	3.23	20.5
31	4227	3		12.3	14.2			9		11.82	2.66	22.5
32	4228	4		10.6	9.52			5.1		8.39	2.92	34.8
33	4230	6		5.34	7.8			4.8		5.99	1.59	26.6
34	4232	8		13.7	7.43			3.6		8.26	5.11	61.9
35	4236	12		12.4	18.3	4		3.1		9.45	7.22	76.4
36	4240	16		19.3	16.9			2.8		13.03	8.92	68.4
37	4244	20		13.3	13.3	1.3		1.5		7.35	6.87	93.5
38	4248	24		8.31	9.27	1.2		0.6		4.84	4.58	94.6

The plasma concentration versus time graphs for ketoconazole following oral administration of 800 mg regimen for all patients are illustrated in figures 3.21 - 3.26.

Twenty seven samples were collected from patient LAA, the plasma concentration versus time is depicted in figure 3.21. Plasma levels started at 0 and accomplished a maximum of 11.88 µg/ml at 3 hrs and then fell to 1.07 µg/ml in 16 hr during the first day. Samples numbers 10 – 15 were not collected. The patient resumed providing blood samples at week 3. From this period till the last day of sampling, the levels fluctuated between blood samples taken pre dose (trough concentration) and post dose (peak concentration. Interestingly, two maxima were observed in the last day where the concentration was raised from 4.62 µg/ml reached a maximum of 15.56 µg/ml in 2 hrs and fall to 5.34 µg/ml. The other maximum was observed (19.34 µg/ml) at 16 hours. Following the second maximum, plasma levels were dropped to 8.31 µg/ml in 24 hours period.

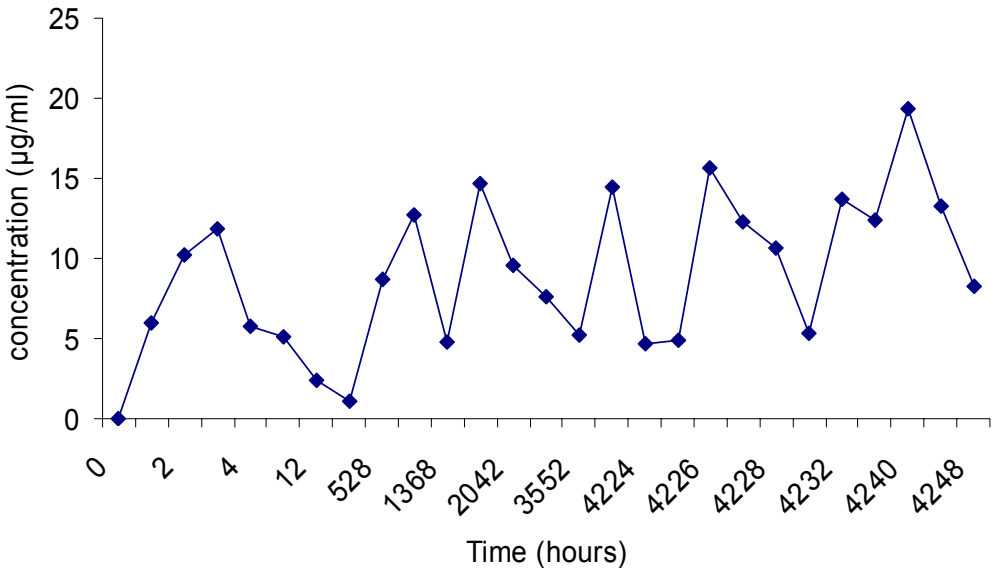

Figure 3.21: Plasma concentration-time profile following administration of 800 mg ketoconazole regimen to patient LAA.

Patient SH showed plasma concentrations that are comparable to the majority of the patients (figure 3.22). His levels started from 0 µg/ml reached a ceiling of 14.68 µg/ml at 2 hrs and then fell to 0.88 µg/ml after 24 hrs. The highest concentration seen after 8 weeks was 13.78 µg/ml. In the last 24 hrs the profile of this patient was observed to similar to that of previous patient (LAA). Concentration increased from 0.72 µg/ml and attained a first peak level of 18.38 µg/ml at 2 hrs and another level of 18.27 µg/ml in 12 hours. This may due to the fact that the two patients took the drug twice instead of once in the last day. The total number of samples collected from this patient was 30 samples.

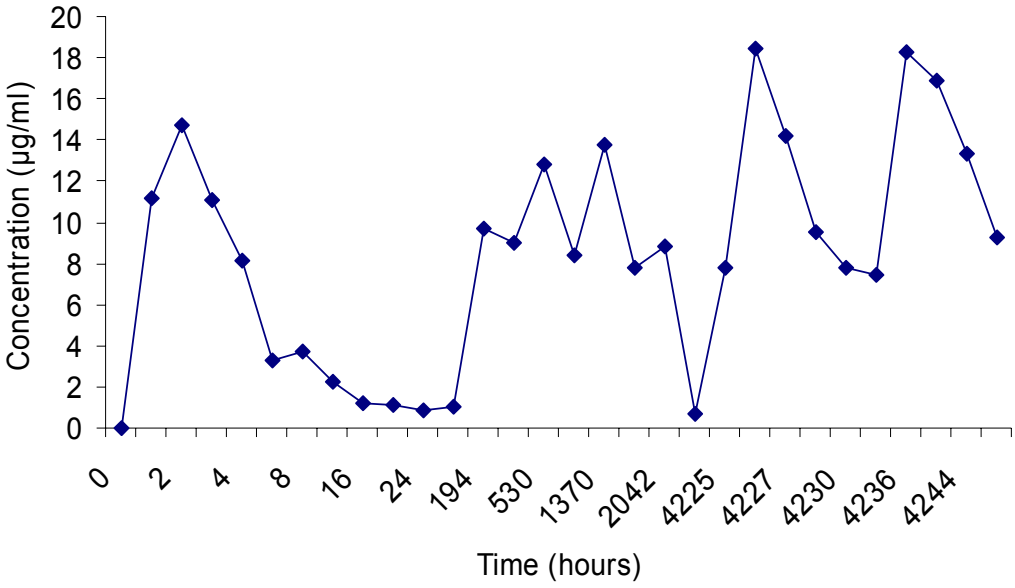

Figure 3.22: Plasma concentration-time profile following administration of 800 mg ketoconazole regimen to patient SH.

Figure 3.23 represents the plasma concentration versus time for 28 plasma samples taken from patient AAO. For the first 24 hrs ketoconazole concentration began at 0 µg/ml reached a maximum of 16.21 µg/ml in 2 hrs and then declined to 0.71 µg/ml in 16 hours. The levels fluctuated between minimum and maximum steady state concentration for the next period; till six months of therapy. The concentration increased after dosing as observed in the last day from 8.84 µg/ml to achieve a peak of 17.81 µg/ml in 2 hrs then decreased to 1.19 in 24 hrs. It has been observed that samples 10 – 12 and 31 – 34 are missing.

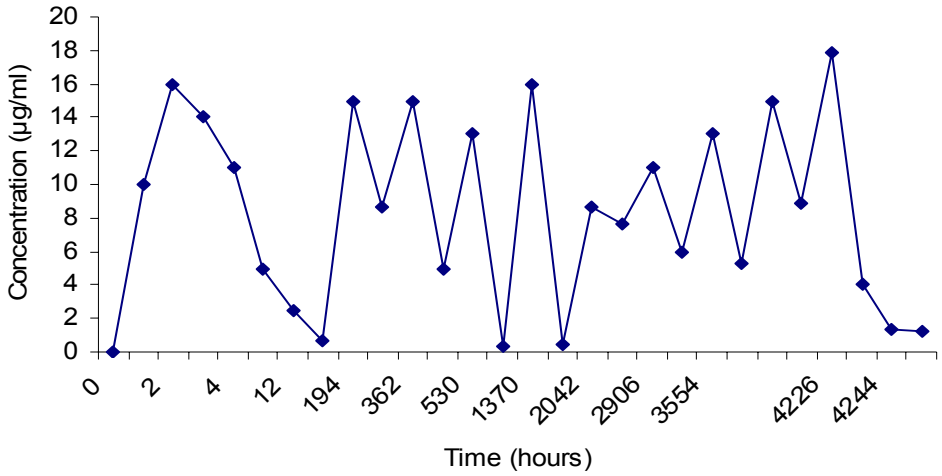

Figure 3.23: Plasma concentration-time profile following administration of 800 mg ketoconazole regimen to patient AAO.

Only 13 plasma samples were obtained from patient SE (figure 3.24) since he dropped out of the study on the second week. This Patient showed the highest ketoconazole concentrations in relation to the other six patients. Following the administration of the first dose, the patient showed maximum concentration of 30.42 µg/ml detected after 2 hrs. The decreased steadily to a level of 0.56 in 24 hours.

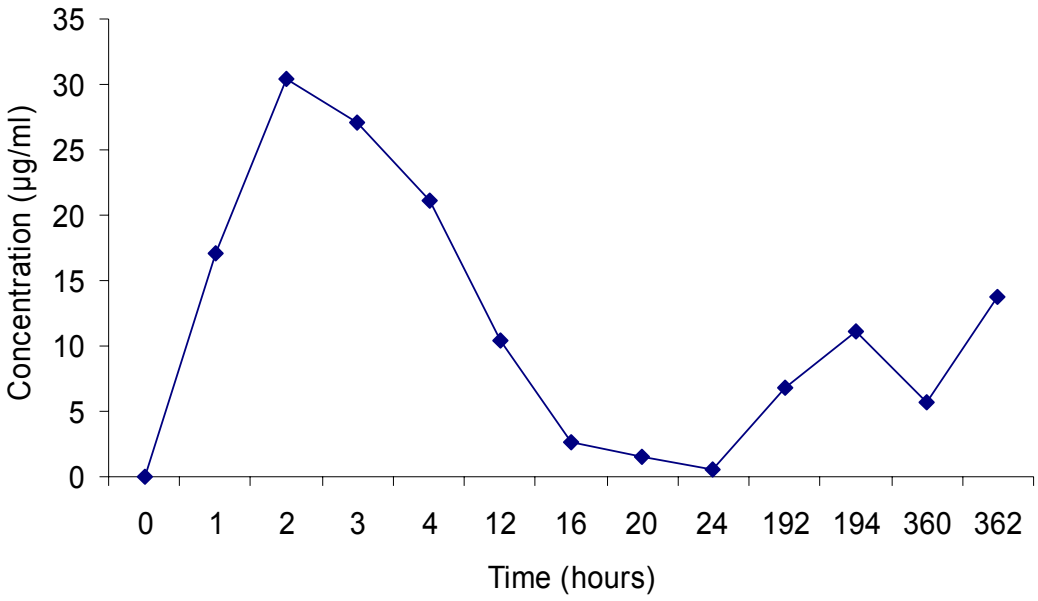

Figure 3.24: Plasma concentration-time profile following administration of 800 mg ketoconazole regimen to patient SE.

Plasma concentration – time profiles for patient AAM is demonstrated in figure 3.25. Thirty three blood samples were obtained, which represent the lowest levels measured among all the patients. The concentration started from 0 µg/ml and achieved 9.38 µg/ml after 2 hrs then decreased to 0.34 µg/ml after 16 hrs. Fluctuation at steady state continued till the 6th month. In the last 24 hrs of sampling schedule, the concentration increased from 0.52 to a maximum of 11.26 µg/ml in 2 hrs then drop dawn to 0.6 µg/ml in 24 hours.

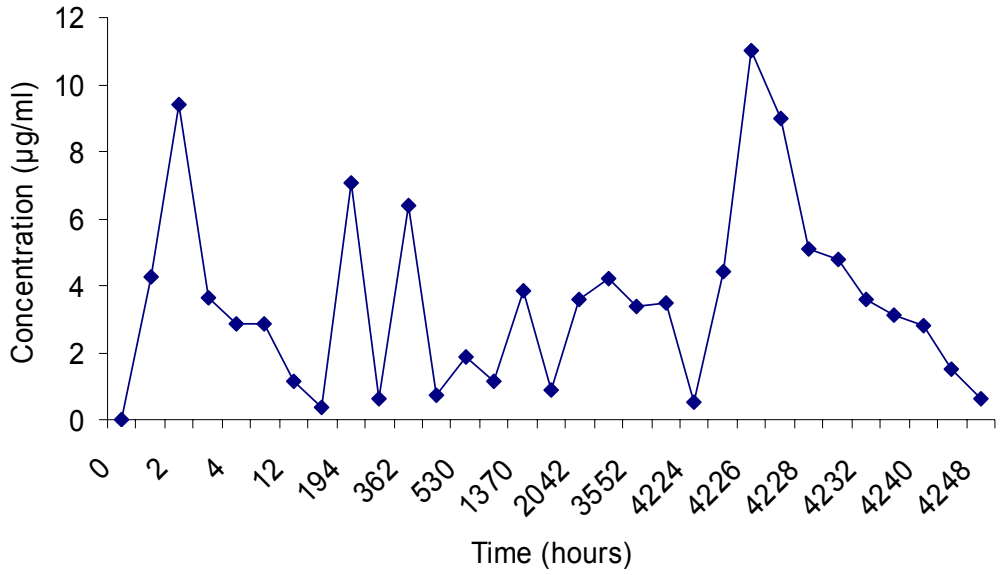

Figure 3.25: Plasma concentration-time profile following administration of 800 mg ketoconazole regimen to patient AAM.

Patient OE attended 2 weeks of sampling only with 14 samples analyzed to obtain the profile drawn in figure 3.26. As can be seen in the profile the maximum concentration of ketoconazole attained by this patient is 20.66 µg/ml observed after 2 hrs following the administration of the first dose. The levels then decrease to 0.41 µg/ml in 24 hours. The profile shows levels fluctuating between trough and peak concentrations for week 1 and 2.

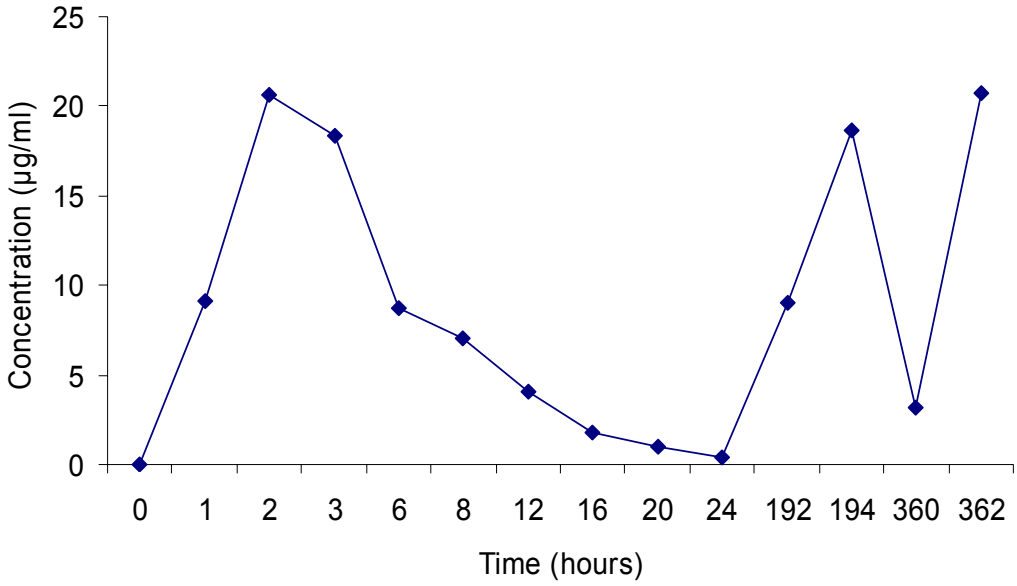

Figure 3.26: Plasma concentration-time profile following administration of 800 mg ketoconazole regimen to patient OE.

A plot of the plasma levels of ketoconazole following its administration to the six patients is shown in figure 3.27. The first part of the profile was consistent with respect to the time to reach maximum concentration.

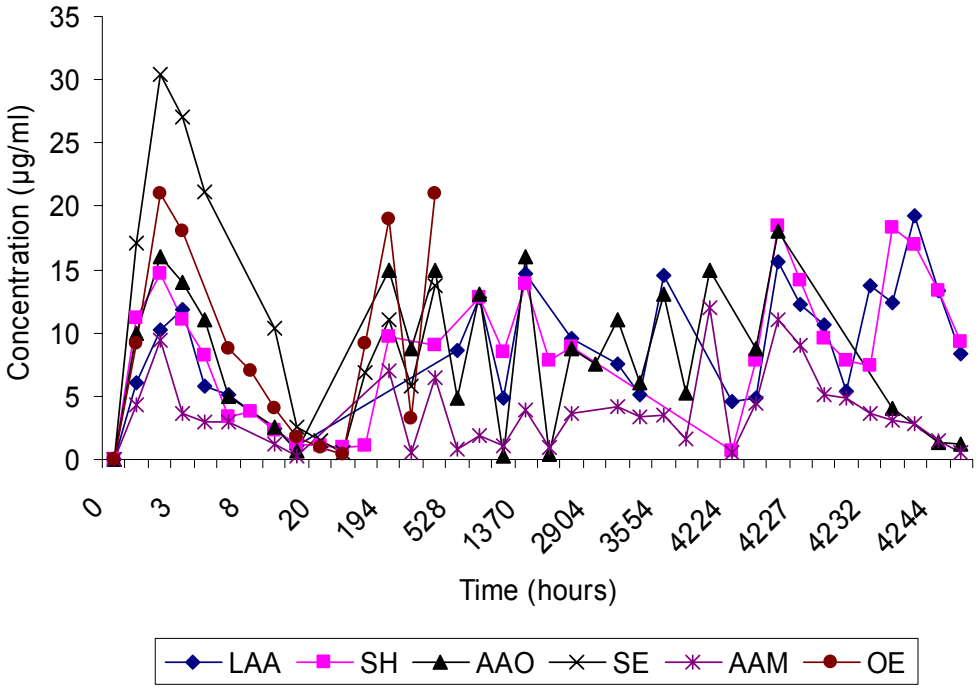

Figure 3.27: Plasma concentration-time profile following administration of 800 mg ketoconazole regimen to 6 mycetoma patients.

Figure 3.28 illustrates the Mean (± SE) plasma concentration versus time following the administration of 800 mg ketoconazole regimen to 6 mycetoma patients. The mean (± SD) maximum concentration attained is 16.92 ± 7.8 µg/ml achieved after 2 hrs. The concentration decreased to 0.62 ± 0.24 after 24 hours. Following the first day of treatment plasma concentration repeatedly decreased and increased till the last day of therapy. Two maxima were observed following the administration of ketoconazole on the last day. The first one occurred after 2 hours 15.75 µg/ml and the second one at 16 hours 13.03 µg/ml following drug administration. The average is greatly influenced by two patients (LAA and SH) who probably ingested the drug 12 hours a part instead of a single 800 mg once.

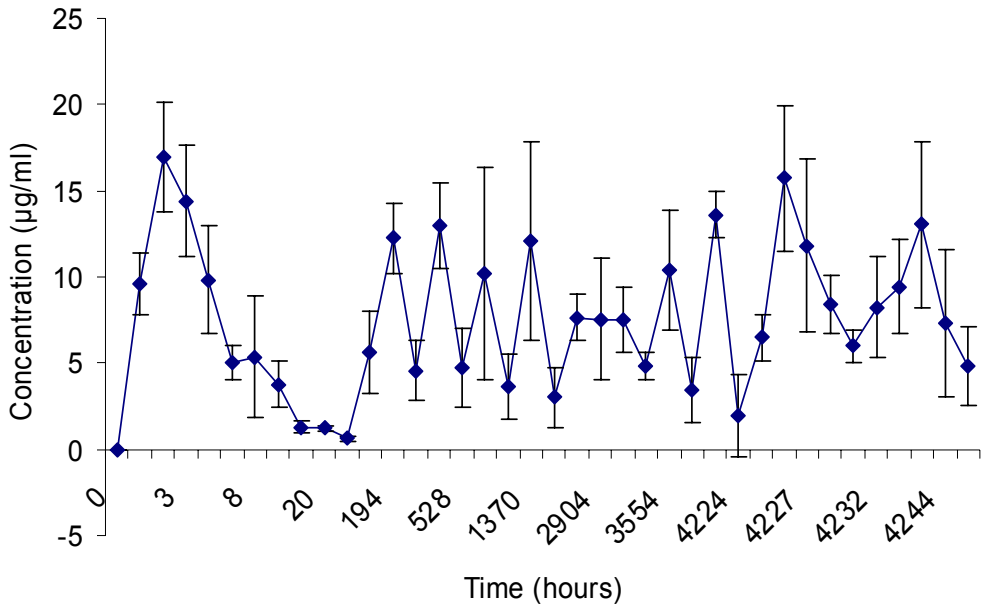

Figure 3.28: Mean (± SE) plasma concentration-time profile following the administration of 800 mg ketoconazole regimen to 6 mycetoma patients

When evaluating the plasma concentration versus time following the administration of the first dose of the 800 mg ketoconazole regimen, the 6 mycetoma patients showed consistent profiles (figure 3.29). Interestingly, the maximum concentration was attained in 2 hours for all the patients except for one (LAA) who achieved maximum level in 3 hours.

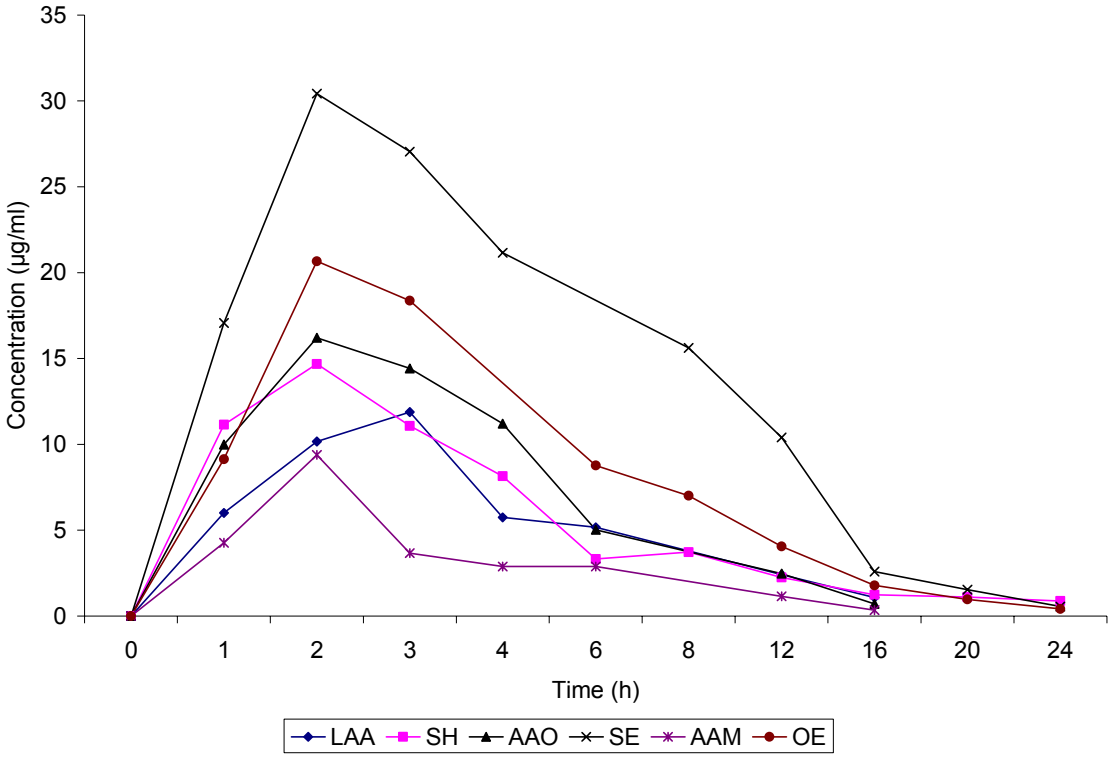

figure 3.29: Plasma concentration - time profiles following the administration of the first dose 800 mg ketoconazole regimen in 6 mycetoma patients

In figure 3.30 the mean (± SE) plasma concentration-time values were plotted following the administration of the first 800 mg dose. Average maximum concentration of 16.92 (± 7.8) µg/ml was achieved after an average T_{max} of 2.17 (± o.41) hrs. The levels then dropped to an average of 0.62 (± 0.24) µg/ml in 24 hours.

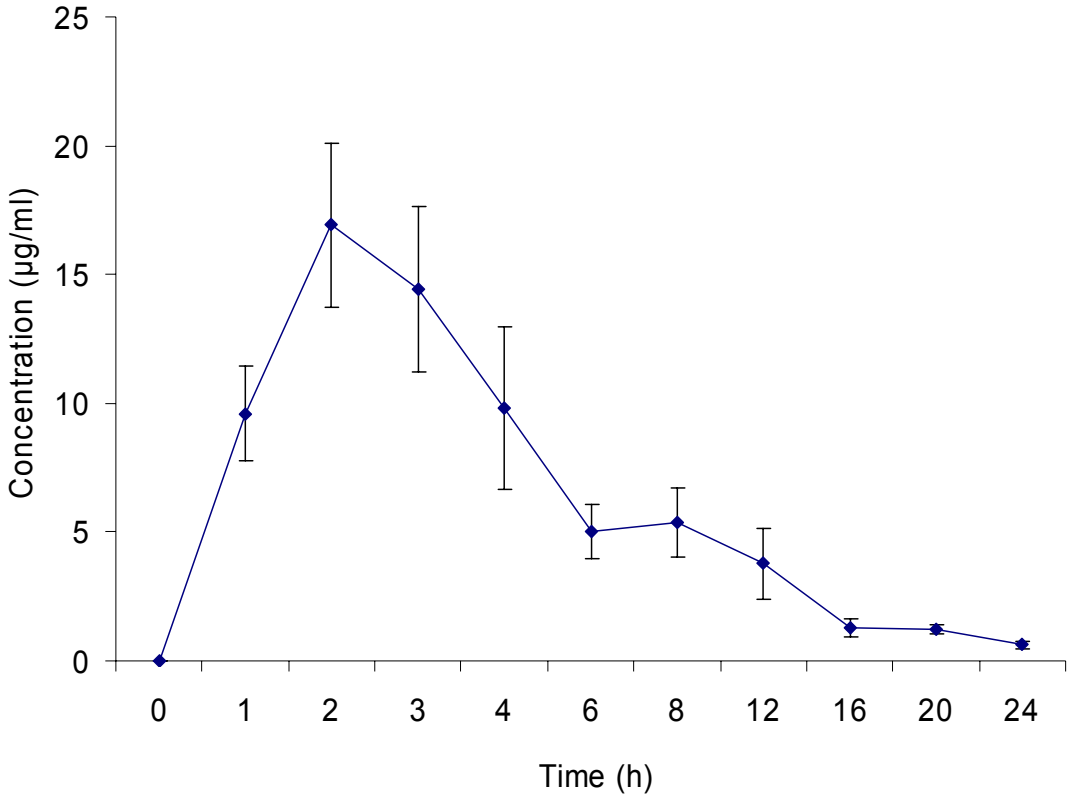

Figure 3.30: Mean (± SE) Plasma concentration - time profiles following the administration of the first dose 800 mg ketoconazole regimen to 6 mycetoma patients

Individual and average (± SE) plasma concentration-time profiles following the administration of the last dose of ketoconazole to Mycetoma patients (n = 4) are depicted in figures 3.31 and 3.32, respectively.

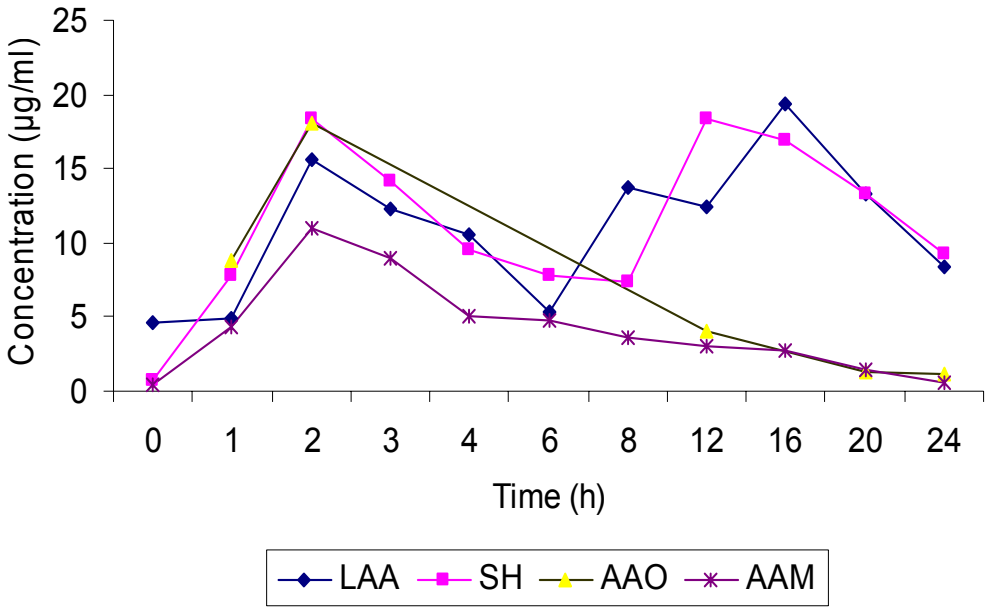

Figure 3.31: Plasma concentration-time profiles following the administration of the last dose 800 mg ketoconazole regimen to 4 mycetoma patients

As can be seen in figure 3.32, the concentration increased from 1.95 ± 2.31 µg/ml reached a maximum of 15.75 ± 3.23 µg/ml after 2 hrs and dropped to 11.82 ± 2.66 µg/ml and increased again to 13.03 ± 8.92 µg/ml after 16 hrs and declined to 4.84 ± 4.58 µg/ml in the last 24 hrs of sampling. The profile seems like a double peak pharmacokinetic phenomena. It is known that ketoconazole kinetics shows these phenomena. The double peaks in serum concentration, seen at higher doses of ketoconazole, raise the question of enterohepatic circulation or delayed absorption (Brass et al, 1982).

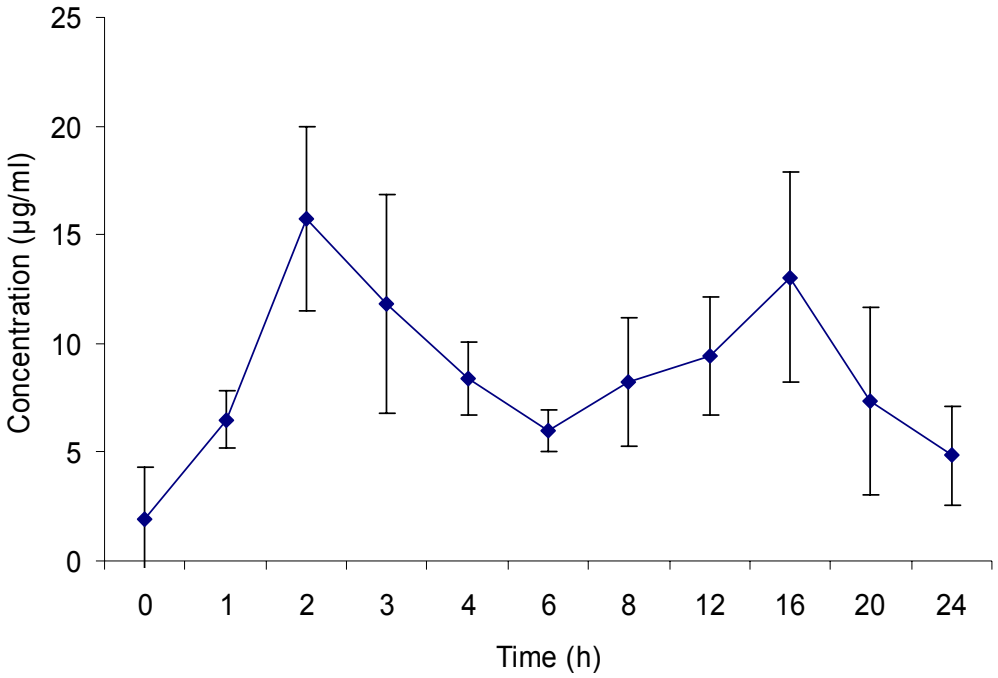

Figure 3.32. Mean (± SE) Plasma concentration - time profiles following the administration of the last dose 800 mg ketoconazole regimen for 4 mycetoma patients

When comparing the concentrations after the first and last doses of the 800 mg ketoconazole regimen (figure 3.33) during the first 6 hours; the first dose achieved higher peak concentration (16.92 ± 7.8 µg/ml) than the last dose (15.75 ± 3.23 µg/ml). The corresponding times to peaks are 2 hours for the two doses.

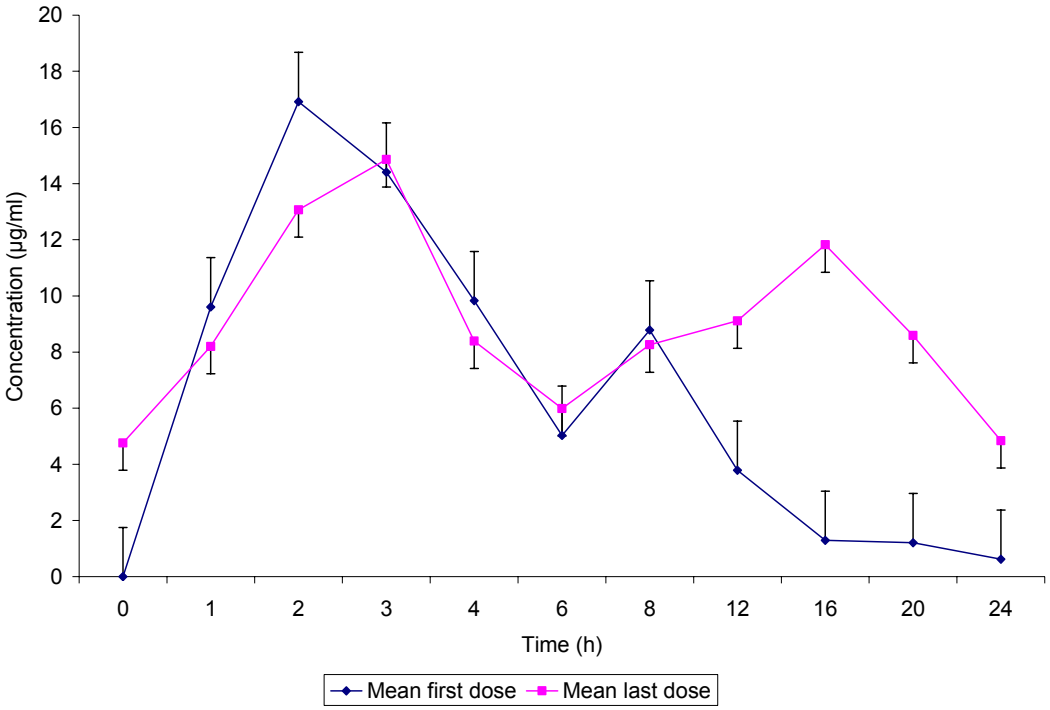

Figure 3.33: Mean (± SE) plasma concentration-time profile following the administration of the first and last dose of 800 mg ketoconazole regimen to mycetoma patients (n = 6)

Figures 3.34 demonstrate the mean (± SE) plasma concentration versus time profiles of ketoconazole for the tow regimens. Concentrations attained when the patients were given the 800 mg ketoconazole regimen were higher compared with 400 mg ketoconazole regimen.

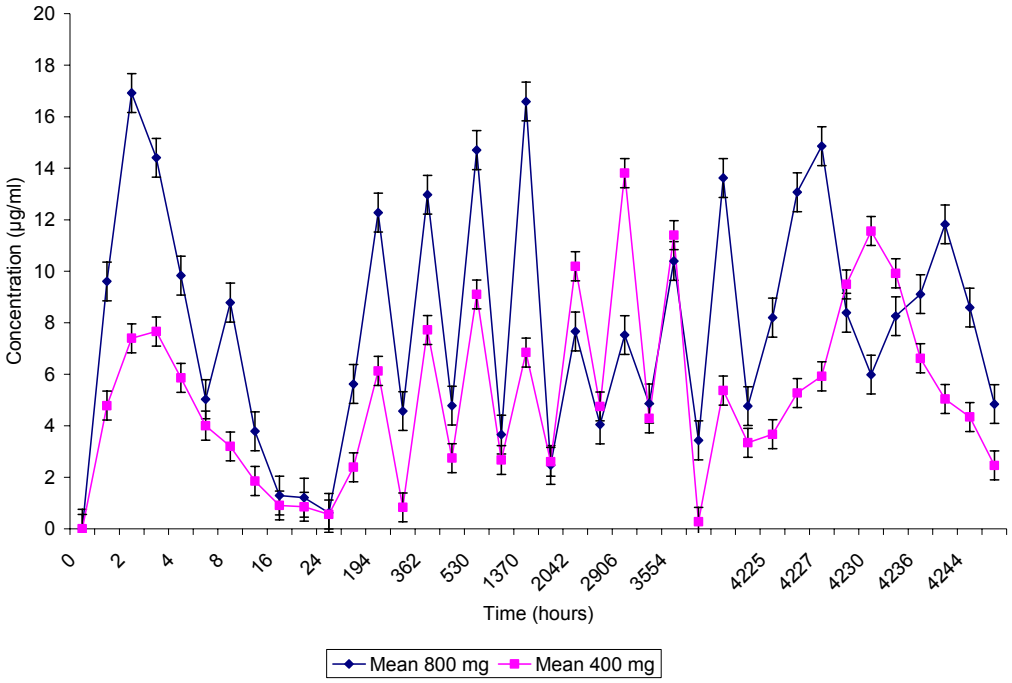

Figure 3.34: Mean (± SE) plasma concentration-time profile following the administration of the 400 mg and 800 mg ketoconazole regimen to 12 mycetoma patients

Tables 3.5 and 3.6 represent the pharmacokinetic parameters of 800 mg ketoconazole tablets following the administration of the first and last doses to six Mycetoma patients, respectively

The mean (± SD) values of maximum drug concentration (Cp_{max}) of 800 mg ketoconazole regimen after administration of first dose was found to be 17.21 ± 7.53 µg/ml (range, 9.38 – 20.65 µg/ml) and that for last dose was 18.44 ± 7.35 µg/ml (range, 11.26 – 28.54 µg/ml). The coefficients of variations (CV %) are 43.77% and 39.84%, respectively.

The mean (± SD) values of maximum time to reach Cp_{max} (T_{max}) for 800 mg ketoconazole regimen after administration of first dose and last dose were found to be 2.17 ± 0.41 h (range, 2 - 3 h, CV% 18.84%) and 2.00 ± 0.82 h (range, 1 - 3 h, CV% 42.82%), respectively.

The mean (± SD) of AUC_{∞} and $AUMC_{\infty}$ following the administration of a single dose of 800 mg ketoconazole are in the sequence of 121.56 ± 71.52 µg.h/ml (range, 41.27 – 252.19 µg.h/ml and CV%, 58.84%) and 959.40 ± 597.39 µg.h2/ml (range, 235.85 – 1775.01 µg.h2/ml and CV%, 62.27%) for first dose and 264.56 ± 136.96 µg.h/ml (range, 87.97 – 378.30 µg.h/ml, CV%, 51.77%) and 3644.23 ± 2930.57 µg.h/ml (range, 998.77 – 6460.04 µg.h/ml, CV%, 80.42%) for last dose. The percentages of AUC_{24}, AUC_{20} and even AUC_{16} of three patients in relation to their AUC_{∞} were 97.7%, 95.4% and 91.6% respectively. This indicates that the estimated areas were less than 10% of the total area. This proofs the accuracy of the proposed sampling schedule.

MRT values (mean ± SD) are found to be 7.86 ± 3.28 h (range, 5.42 - 14.28 h) and 12.49 ± 5.29 h (range, 5.49 - 17.08 h) following the administration of first and last doses. The corresponding coefficients of variations are 41.73% and 42.34%.

The estimated mean (± SD) values of CL/F were 8.79 ± 5.57 L/h (range, 3.17 – 19.38 L/h, CV% = 63.44%) and 3.18 ± 2.48 L/h (range, 1.59 - 6.82 L/h, CV% = 78.03%) after first dose and last dose, respectively.

The mean (± SD) values of Vd/F were in the order of 49.28 ± 27.11 L (range, 17.23 – 96.61 L, CV%, 55.01%) and 36.66 ± 40.14 L (range. 10.91 – 96.27 L, CV%, 109.51%) after 800 mg first dose and 800 mg last dose.

The calculated mean (± SD) values of the elimination half - life ($T_{1/2}$) of ketoconazole were 4.00 ± 0.66 h (range, 3.23 – 4.98 h, CV%, 16.45%) and 6.83 ± 2.15 (range, 4.63 – 9.78 h, CV%, 31.49%) following the first dose and last doses, respectively.

Table 3.5 ketoconazole pharmacokinetic parameters after oral administrations of first dose 800 mg to 6 patients.

Patient	Parameter											
	T_{max} (h)	Cp_{max} (µg/ml)	AUC∞ (µg.h/ml)	AUMC ($\mu g.h^2/ml$	MRT (h)	K_e (h^{-1})	$t_{1/2}$ (h)	CL/F (L/h)	Vd/F (L)	AUC24 (µg.h/ml)	AUC20 (µg.h/ml)	AUC16 (µg.h/ml)
LAA	3.00	11.88	108.55	1550.34	14.3	0.14	4.98	7.37	52.91			71.505
SH	2.00	14.68	92.77	744.09	8.02	0.15	4.50	8.62	56.03	87.06	83.075	78.375
AAO	2.00	16.21	94.59	512.59	5.42	0.21	3.23	8.46	39.37			91.285
SE	2.00	30.42	252.1913	1775.018	7.04	0.18	3.76	3.17	17.23	249.15	244.95	236.69
AAM	2.00	9.38	41.27	235.85	5.71	0.20	3.45	19.38	96.61			39.58
OE	2.00	20.66	139.96	938.48	6.71	0.17	4.06	5.72	33.51	137.5 6	134.795	129.275
Mean	2.17	17.21	121.56	959.40	7.86	0.18	4.00	8.79	49.28	157.92	154.27	107.79
SD	0.41	7.53	71.52	597.39	3.28	0.03	0.66	5.57	27.11	82.94	82.68	69.55
CV%	18.89	43.75	58.84	62.27	41.7	16.67	16.5	63.37	55.01	52.52	53.59	18.89

Table 3.6 ketoconazole pharmacokinetic parameters after oral administrations of last dose 800 mg to 4 patients

Patient	Parameter											
	T_{max} (h)	Cp_{max} (µg/ml)	AUC∞ (µg.h/ml)	AUMC ($\mu g.h^2/ml$	MRT (h)	K_e (h^{-1})	$t_{1/2}$ (h)	CL/F (L/h)	Vd/F (L)	AUC24 (µg.h/ml)	AUC20 (µg.h/ml)	AUC16 (µg.h/ml)
LAA	2.00	15.56	378.30	6460.04	17.08	0.11	6.56	1.59	15.02	299.60	256.38	191.10
SH	2.00	18.38	367.30	5885.31	16.02	0.15	4.63	1.63	10.91	305.38	250.30	187.18
AAO	3.00	28. 54	224.65	1232.82	5.49	0.11	6.34	2.67	24.42	213.77	208.71	187.35
AAM	1.00	11.26	87.97	998.77	11.35	0.07	9.78	6.82	96.27	79.50	75.40	66.84
Mean	2.00	18.44	264.56	3644.23	12.49	0.11	6.83	3.18	36.66	224.56	197.70	158.12
SD	0.82	7.35	136.96	2930.57	5.29	0.03	2.15	2.48	40.14	105.39	84.24	60.88
CV%	41.0	39.9	51.8	80.4	42.4	27.3	31.5	78.0	109.5	46.9	42.6	41.0

Table 3.7: Mean (± SD) pharmacokinetic parameters of Ketoconazole for the tow regimen

Parameter	400 mg 1st dose	*P* - value*	400 mg last dose**	800 mg 1st dose	800 mg last dose	*P* - value ***
T_{max} (h)	2.33 ± 0.52	0.18	6.00	2.17 ± 0.41	2.00 ± 0.82	0.36
C_{max} (µg/ml)	9.08 ± 3.96	0.04	11.56	17.21 ± 7.53	18.44 ± 7.35	0.40
AUC∞ (µg.h/ml)	62.34 ± 31.03	0.05	183.07	121.56 ± 71.52	264.56 ± 136.96	0.06
AUMC ($\mu g.h^2/ml$)	498.12 ± 270.79	0.02	2681.67	95940 ± 597.39	3644.23 ± 2930.57	0.08
MRT (h)	8.15 ± 1.58	0.43	14.65	7.86 ± 3.28	12.49 ± 5.29	0.09
K_e (h^{-1})	0.21 ± 0.14	0.28	0.08	0.18 ± 0.03	0.11 ± 0.03	0.01
$T_{1/2}$ (h)	4.24 ± 1.87	0.38	8.60	4.00 ± 0.66	6.83 ± 2.15	0.04
CL/F (L/h)	8.78 ± 6.85	0.5	1.09	8.79 ± 5.57	3.18 ± 2.48	0.03
Vd/F (L)	42.70 ± 14.67	0.34	13.55	49.28 ± 27.11	36.66 ± 40.14	0.30
AUC 24 (µg.h/ml)	46.46 ± 25.81	0.21	152.57	157.92 ± 82.94	244.56 ± 105.39	0.20

* Estimated p value for 400 mg 1st dose and 800 mg 1st dose

** Values obtained from only one patient.

*** Estimated p value for 800 mg 1st dose and 800 mg last dose

3.4. Comparisons of pharmacokinetic parameters of ketoconazole following administration of the two regimens

Comparisons of pharmacokinetic parameters of ketoconazole following its administration as 400 mg and 800 mg regimens are shown in table 3.7.

3.4.1. Comparisons following the administration of the first dose of the two regimens

The peak concentrations achieved after 400 mg (9.08 ± 3.96 µg/ml) is almost double that following the administration of 800 mg ketoconazole (17.21 ± 7.53 µg/ml). On the other hand, the mean times to reach Cp_{max} (T_{max}) were comparable between the two dosing regimens (2.33 ± 0.52 and 2.17 ± 0.41, respectively).

The mean values of AUC_{∞} are found to be 62.34 ± 32.03 µg.h/ml for 400 mg regimen and 121.56 ± 71.52 µg.h/ml for 800 mg regimen. While the estimated values of $AUMC_{\infty}$ were 498.12 ± 270.79 $\mu g.h^2$/ml and 959.4 ± 597.39 $\mu g.h^2$/ml.

The obtained values for MRT, Ke and $t_{1/2}$ were 8.15 ± 1.58 and 7.86 ± 3.28 h; 0.21 ± 0.14 and 0.18 ± 0.03 h; 4.24 ± 1.87 and 4 ± 0.66 h for 400 and 800 mg regimens, respectively.

The physiological parameters Cl/F and Vd/F were estimated as 8.78 ± 6.85 and 8.79 ± 5.57 L/h; 42.7 ± 14.67 and 49.28 ± 27.11 L for the two regimens.

3.4.2. Comparisons following the administration of the last dose of the two regimens

The data obtained following the last dose was compared between the mean of four patients in the 800 mg regimen and only one patient in the 400 mg regimen.

The peak concentrations reached following the administration of 400 mg ketoconazole were 11.56 µg/ml compared to that of 800 mg ketoconazole (18.44 ± 7.35 µg/ml). The values of T_{max} between the two dosing regimens were 6.0 and 2.00 ± 0.82, respectively.

The mean values of AUC_{∞} are found to be 183.07 µg.h/ml for 400 mg regimen and 264.56 ± 136.96 µg.h/ml for 800 mg regimen. While the estimated values of $AUMC_{\infty}$ were 2681.67 $\mu g.h^2/ml$ and 3644.23 ± 2930.57 $\mu g.h^2/ml$.

The calculated parameters MRT, Ke and $t_{1/2}$ were found to be 14.65 and 12.49 ± 5.29 h; 0.08 and 0.11 ± 0.03 h; 8.6 and 6.83 ± 2.15 h for 400 and 800 mg regimens, respectively.

The physiological parameters Cl/F and Vd/F were estimated as 1.09 and 3.18 ± 2.48 L/h ; 13.55 and 36.66 ± 40.14 L for the two regimens, respectively.

3.4.3. Comparisons of steady state levels

Plasma concentration fluctuated between minimum steady state concentrations (Cp_{ssmin}) of 2.68 ± 1.40 (CV%, 52.13%) and 4.18 ± 0.98 (CV%, 23.52%) and maximum steady state concentrations (Cp_{ssmax}) of 8.00 ± 1.65 (CV%, 20.67) and 11.97 ± 3.24 (CV%, 27.07%) following the administration of the 400 mg and 800 mg ketoconazole regimens, respectively.

The corresponding fluctuation at steady state (F, calculated as Cp_{ssmax}/Cp_{ssmin}) was found to be 3.98 ± 3.01 for the 400 mg regimen and 2.96 ± 0.93 for the 800 mg regimen

Table 3.8 Steady state pharmacokinetic of ketoconazole following its administration as 4oo and 800 mg regimens

	Dose					
	400 MG			800 MG		
	trough Cp	peak Cp	fluctuation	trough Cp	peak Cp	fluctuation
Concentration	2.39	6.13	2.56	5.62	12.28	2.19
	0.83	7.72	9.30	4.57	12.97	2.84
	2.75	9.10	3.31	4.78	14.71	3.08
	2.67	6.84	2.56	3.66	16.59	4.53
	4.75	10.19	2.15	2.48	7.66	3.09
	-	-	-	4.05	7.52	1.86
	-	-	-	4.86	10.40	2.14
	-	-	-	3.44	13.62	3.96
Mean	2.68	8.00	3.98	4.18	11.97	2.96
SD	1.40	1.65	3.01	0.98	3.24	0.93
CV%	52.13	20.67	75.60	23.52	27.08	31.30

4. Discussion

There are many studies on pharmacokinetics of ketoconazole preparations in healthy volunteers and patients. There are also some animal studies. Some objectives of these studies were to assess the clinical use and adverse effects of ketoconazole. In the present study the pharmacokinetics and clinical efficacy of two dosing regimens of ketoconazole in eumycetoma patients were investigated.

4.1. Analytical procedure

Different analytical procedures were used to determine the concentration of ketoconazole in biological fluids. In this study we evaluated the pharmacokinetic parameters and bioavailability by means of HPLC. The HPLC procedure was specific, sensitive and selective for the determination of ketoconazole and itraconazole the internal standard in plasma. Reasonable retention times were achieved for ketoconazole (2.03 minutes) and itraconazole (5.23 minutes) with a total run time of 6 minutes. Chromatograms showed no interfering peaks and good regression (R2 = 0.992705) of the calibration curves was achieved. The limit of quantification was 50 ng/ml.

Some patients did not comply with the protocol of the study. They did not complete the sampling schedule as specified and dropped out at different periods due to different reasons. Some of the patients live in areas far from the hospital with low economical and social status. They are mostly farmers, cultivators, wood cutters and herdsmen. The nature of long-term treatment and continuous follow up is new for those patients. They tend to discontinue the treatment and search other remedies e.g. cauterization and/or traditional medicine. Nonetheless, treatment of patients who dropped for other reasons was continued. It has been observed that some samples were also missing. This may be during sampling, storage or analysis. Some outlier values were observed that we do not have explanation for and were included in the analysis. Interpolation of data was performed regarding the missing samples.

Table 4.1 Reported values of ketoconazole pharmacokinetic parameters

Pharmacokinetic Parameter (Mean ±SD)	n	Dose	Method of analysis	Value	Reference	Species
$T_{max\ (h)}$	24	400 mg	HPLC	2.02 ± 0.18*	Nizoral® Monograph	Human
		200 mg	HPLC	2.62 ± 0.91	Daneshmend et al. (1984)	Human
		400 mg	HPLC	3.00± 0.76	Daneshmend et al. (1984)	Human
		800 mg	HPLC	2.85± 1.07	Daneshmend et al. (1984)	Human
	4	10 mg/kg	EEG	0.50 ±0.29	Kotegawa et al. (1999)	Rat
		200 mg	HPLC	1.40	Mannisto et al. (1982) Fasting	Human
		200 mg	HPLC	2.30	Mannisto et al. (1982) Meals	Human
		200 mg	HPLC	1.80	Mannisto et al. (1982) Orange juice	Human
	160	400 mg	HPLC	4.00 – 6.00*	Sugar et al. (1987)	Human
	24	200 mg	HPLC	1.00 – 4.00*	Huang et al. (1986)	Human
	6	400 mg	HPLC	2.33 ± 0.52	The present study	Human
	6	800 mg	HPLC	2.17 ± 0.41	The present study	Human
C_{max} (µg/ml)		200 mg	HPLC	3.29 ± 0.22	Daneshmend et al. (1984)	Human
	9	200 mg	HPLC	3.20 ± 1.80	Stockley et al. (1986)	Human
		200 mg	HPLC	4.10 ± 0.30*	Mannisto et al. (1982) Fasting	Human
		200 mg	HPLC	2.30 ± 0.30*	Mannisto et al. (1982)Meals	Human
		200 mg	HPLC	3.60 ± 0.20*	Mannisto et al. (1982)Orange juice	Human
		200 mg	HPLC	1.54 – 3.12*	Negroni et al. (1980)	Human
		200 mg	HPLC	1.60 – 7.00*	Robertson et al. (1980)	Human
		200 mg	HPLC	2.50	Hay et al. (1980)	Human
		200 mg	HPLC	1.56 – 3.12*	Galimberti et al. (1980)	Human
		200 mg	HPLC	3.50	Gascoigne et al. (1981)	Human

		200 mg	HPLC	1.50–4.50*	Huang et al. (1986)	Human
		400 mg	HPLC	10.64 ± 0.73	Daneshmend et al. (1984)	Human
	9	400 mg	HPLC	4.60 ± 3.20	Stockley et al. (1986)	Human
		400 mg	HPLC	3.42	Espinel – Ingraff et al. (1981)	Human
		400 mg	HPLC	6.50	Daneshmend et al. (1981)	Human
		400 mg	HPLC	5.50	Gascoigne et al. (1981)	Human
	160	400 mg	HPLC	7.00 – 17.00*	Sugar et al. (1987)	Human
		800 mg	HPLC	19.22 ± 1.99	Daneshmend et al. (1984)	Human
	24	400 mg	HPLC	6.65 ± 0.763*	Nizoral® Monograph	Human
	4	10 mg/kg	EEG	9.20 ± 4.50*	Kotegawa et al. (1999)	Human
	6	400 mg	HPLC	9.08 ± 3.96	The present study	Human
	6	800 mg	HPLC	17.21 ± 7.53	The present study	Human
AUC∞ (µg.h/ml)		200 mg	HPLC	13.6 ± 1.2	Daneshmend et al. (1984)	Human
	9	200 mg	HPLC	12.30 ± 7.70	Stockley et al. (1986)	Human
		400 mg	HPLC	59.2 ± 6.20	Daneshmend et al. (1984)	Human
	9	400 mg	HPLC	23.00 ± 18.20	Stockley et al. (1986)	Human
		800 mg	HPLC	151.20 ± 16.60	Daneshmend et al. (1984)	Human
		200 mg	HPLC	14.40 ± 2.21*	Mannisto et al. (1982) Fasting	Human
		200 mg	HPLC	8.60 ± 1.33*	Mannisto et al. (1982)Meals	Human
		200 mg	HPLC	13.40 ± 1.30*	Mannisto et al. (1982)Orange juice	Human
	24	400 mg	HPLC	46.161 ± 7.135*	Nizoral®	Human
	4	10 mg/kg	EEG	26.80 ± 15.70*	Kotegawa et al. (1999)	Rat
	6	400 mg	HPLC	62.34 ± 31.03	The present study	Human
	6	800 mg	HPLC	121.56 ± 71.52	The present study	Human

$T_{1/2}$ (h)		200 mg	HPLC	1.43 ± 0.08	Daneshmend et al. (1984)	Human
	9	200 mg	HPLC	3.10 ± 1.90	Stockley et al. (1986)	Human
		400 mg	HPLC	2.45 ± 0.27	Daneshmend et al. (1984)	Human
	9	400 mg	HPLC	3.50 ± 1.70	Stockley et al. (1986)	Human
		800 mg	HPLC	3.62 ± 0.20	Daneshmend et al. (1984)	Human
		200 mg	HPLC	1.70 – 2.00*	Mannisto et al. (1982)	Human
		200 mg	HPLC	2.00 – 3.30*	Brass et al. (1982)	Human
		200 mg	HPLC	3.30	Badcock et al. (1987)	Human
	24	200 mg		7.50 – 7.90*	Huang et al. (1986)	Human
	24	400 mg	HPLC	3.47 ± 0.40*	Nizoral® Monograph	Human
	4	10 mg/kg	EEG	1.38 ± 0.52*	Kotegawa et al. (1999)	Rat
	6	400 mg	HPLC	4.24 ± 1.87	The present study	Human
	6	800 mg	HPLC	4.00 ± 0.66	The present study	Human
CL/F (L/h)	4	10 mg/kg	EEG	0.45 ± 0.18	Kotegawa et al. (1999)	Rat
	6	400 mg	HPLC	8.78 ± 6.85	The present study	Human
	6	800 mg	HPLC	8.79 ± 5.57	The present study	Human
Vd/F (L)	4	10 mg/kg	EEG	0.89 ± 0.57	Kotegawa et al. (1999)	Rat
	6	400 mg	HPLC	42.70 ± 14.67	The present study	Human
	6	800 mg	HPLC	49.28 ± 27.11	The present study	Human

* Mean (± SE)

4.2. Pharmacokinetics of ketoconazole tablets; 400 mg regimen

The mean (± SD) values of maximum drug concentration (Cp_{max}) after administration of first dose of 400 mg ketoconazole regimen was found to be 9.08 ± 3.96 µg/ml, while that for last dose was 11.56 µg/ml. Our results were comparable to the values reported by Daneshmend et al, 1984 (10.64 ± 0.73 µg/ml) and Sugar et al, 1987 (7.00 – 17.00 µg/ml) who used the same dose (Table 4.1).

The mean values of time to reach Cp_{max} (T_{max}) after administration of first dose 2.33 ± 0.52 h, range (2 - 3) h and last dose 6 h of 400 mg regimen are comparable with the values (Mean ± SE) of 2.02 ± 0.18 h reported in the Nizoral® Monograph, (1994) and 3.00 ± 0.76 h (Mean ± SD) reported by Daneshmend et al, (1984) .

The mean value of AUC_{∞} for ketoconazole obtained after oral administration of first dose of 400 mg regimen was 62.34 ± 31.03 µg.h/ml (range 17.82 – 113.83 µg.h/ml). And that obtained after oral administration of last dose was 183.07 µg.h/ml. The values of AUC_{∞} reported in the literature (Mean ± SD) using the same dose are 46.161 ± 7.14 µg.h/ml (Nizoral® Monograph, 1994), 59.2 ± 6.2 µg.h/ml (Daneshmend et al, 1984) and 23.00 ± 18.2 µg.h/ml (Stockley et al, 1986). Our results agree with the first two authors and show discrepancy with the last author.

The mean values of CL/F for 400 mg ketoconazole regimen are 8.78 ± 2.80 L/h (range 3.51 ± 22.45 L/h) and 1.09 L/h for first dose and last dose, respectively. The mean values of Vd/F for ketoconazole are in the sequence of 42.70 ± 5.99 L (range 22.47 – 49.35 L) and 13.55 L for first dose and last dose of 400 mg regimen. The values of clearance and volume of distribution reported by Kotegawa et al, (1999) were 0.45 ± 0.18 L/h/kg and 0.89 ± 0.57 L, respectively. The dose used by those authors is of 10 mg/kg in rat. Inter-species differences is possible.

The mean CL/F, calculated by using the AUC from a 200 mg ketoconazole solution, was 209.9 (± 82.9) ml/min, and the mean V/F was 88.31 (± 68.72) liters, suggesting an extensive distribution of the drug in the body (Daneshmend et al.1983). The same author showed that the mean CL/F decreased as the dose increased. It decreased from 244.9 (+96.0) to

123.6 (± 31.4) to 80.8 (± 17.8) ml/min as the dose increased from 200 to 400 to 800 mg, respectively; the differences in the CL/F between the 200 and 800 mg doses and between the 200- and 400 mg doses were statistically significant

The mean values of the elimination half - life ($T_{1/2}$) for ketoconazole are 4.24 ± 1.87 h (range 1.45 – 7.04 h) and 8.60 h for first dose and last dose of 400 mg regimen. These values are in good agreement with a value of 3.5 ± 1.70 reported by Stockley et al, (1986). However, they are lower than the value of 7.50 – 7.90 reported by Huang et al, (1986).

4.3 Pharmacokinetics of ketoconazole tablets; 800 mg regimen

For 800 mg ketoconazole regimen the mean values of Cp_{max} after administration of first dose were found to be 17.21 ± 7.53 µg/ml (range 9.38 – 20.65 µg/ml) and those after last dose were 18.44 ± 7.35 µg/ml (range 11.26 – 28.54 µg/ml). The values of these parameters are almost the same following the administration of the two doses. The values of Cp_{max} obtained for 800 mg ketoconazole regimen, agrees with previous observations (19.22 ± 1.99 µg/ml) reported by Daneshmend et al, (1984).

The mean values of time to reach Cp_{max} (T_{max}) after administration of first dose 2.17 ± 0.41 h, range (2 - 3) h and last dose 2.00 ± 0.82 h, range 1 - 3 h of 800 mg regimen are comparable of 2.85 ± 1.07 h reported by Daneshmend et al, (1984) following the use of the same dose.

The values (Mean ± SD) of AUC_{∞} for 800 mg ketoconazole regimen are in the sequence of 121.56 ± 71.52 µg.h/ml, range 41.27 – 252.19 µg.h/ml for first dose and 264.56 ± 136.96 µg.h/ml, range 87.97 – 378.30 µg.h/ml for last dose. The values reported in the literature are 151.2 ± 16.6 µg.h/ml (Daneshmend et al, 1984).

The MRT for 800 mg ketoconazole regimen was 7.86 ± 3.28 h (range 5.42 - 14.28 h) and 12.49 ± 5.29 h (range 5.49 - 17.08 h) for first and last doses, respectively.

The mean values of CL/F for 800 mg ketoconazole regimen are 8.79 ± 5.57 (range 3.17 – 19.38 L/h) and 3.18 ± 2.48 (range 1.59 - 6.82) L/h following first and last doses, respectively.

The mean values of Vd/F are in the order of 49.28 ± 27.11 (range 17.23 – 96.61) L and 36.66 ± 40.14 (range 10.91 – 96.27) L for first and last doses of 800 mg regimen.

The mean values of the elimination half - life ($T_{1/2}$) for ketoconazole are 4.00 ± 0.66 (range 3.23 – 4.98) h and 6.83 ± 2.15 (range 4.63 – 9.78) h for first dose and last dose 800 mg regimen, respectively. The values reported in the literature are 3.5 ± 1.70 (Stockley et al. 1986), 7.50 – 7.90 (Huang et al, 1986) and 3.62 ± 0.2 h (Daneshmend et al, 1984)

4.4. Comparisons of pharmacokinetic parameters of ketoconazole following administration of the two regimens

Comparisons of pharmacokinetic parameters of ketoconazole following its administration as 400 mg and 800 mg regimens are shown in table 3.7.

4.4.1. Comparisons following the administration of the first and last doses of 400 mg regimen

The maximum plasma concentrations achieved after first dose and last dose were 9.08 ± 3.96 and 11.56 µg/ml, respectively. The corresponding mean values of T_{max} were found to be 2.33 ± 0.52 and 6.0 h for the two dosing regimens. The values of Cp_{max} are comparable; however, those of T_{max} are different. Statistical analysis was not performed since the data of the last dose is obtained from only one patient.

The mean values of AUC_{∞} were found to be 62.34 ± 32.03 µg.h/ml for first dose and 183.08 µg.h/ml for last dose.

The values for $t_{1/2}$ were calculated as 4.24 ± 1.87 and 8.6 h following the administration of first and last doses of 400 mg, respectively. The $t_{1/2}$ increased following the administration of the last dose.

The values of Cl/F and Vd/F were estimated as 8.78 ± 6.85 and 1.09 L/h; 42.7 ± 14.67 and 13.55 L for the two doses. Both clearance and volume of distribution were decreased following the administration of the last dose.

4.4.2. Comparisons following the administration of the first and last doses of 800 mg regimen

The peak concentrations reached after the administration of the first 800 mg dose was 17.21 ± 7.53 µg/ml compared to 18.44 ± 7.35 µg/ml obtained after the last dose. There were no statistically significant differences in Cp_{max} between the tow doses (p = 0.4). The mean values of T_{max} were found to be comparable between the two doses 2.17 ± 0.41 and 2.00 ± 0.82, respectively. There is no statistically significant difference in T_{max} between the two doses (P = 0.36). These values are comparable with values reported in the literature.

The mean values of AUC_{∞} were found to be 121.56 ± 71.52 µg.h/ml for first dose and 264.56 ± 136.96 µg.h/ml for last dose. These values were tested statistically and no significant differences were observed (P = 0.06).

The values for $t_{1/2}$ were calculated as 4 ± 0.66 h and 6.83 ± 2.15 h following the administration of 800 mg as first and last doses, respectively. Statistically significant differences were observed in $t_{1/2}$ following repeated dose administration (P = 0.04). Our findings are in good agreement with the values (7.5 – 7.90 h) reported by Huang et al, (1986) who suggested a dose-dependent increase in half life with increase of the dose and repeated ketoconazole administration.

The physiological parameters Cl/F and Vd/F were estimated as 8.79 ± 5.57 and 3.18 ± 2.48 L/h; 49.28 ± 27.11 and 36.66 ± 40.14 L following the administration of the first and last doses of 800 mg regimen. Decrease in both clearance and volume of distribution was observed following repeated dosing. These differences were tested statistically. No statistically significant differences were observed in volume of distribution. While the differences in clearance were found to be statistically significant between the two doses (P = 0.03). Our results are in good agreement with the results published by Heel and coworkers (1982) who suggested an autoinhibition of ketoconazole metabolism on its long-term treatment in humans.

4.4.3. Comparisons following the administration of the first doses of 400 and 800 mg regimens

The peak concentrations achieved after 400 mg (9.08 ± 3.96 µg/ml) is almost double that following the administration of 800 mg ketoconazole (17.21 ± 7.53 µg/ml). This indicates that ketoconazole follow linear pharmacokinetic behavior at this dosing range. The mean values of T_{max} were found to be comparable between the two dosing regimens (2.33 ± 0.52 and 2.17 ± 0.41, respectively). There is no statistically significant difference in T_{max} between the two doses (P = 0.18). These values are comparable with values reported in the literature by Nizoral® Monograph, (1994) and Daneshmend et al, (1984)

The mean values of AUC_{∞} were found to be 62.34 ± 31.03 µg.h/ml for 400 mg regimen and 121.56 ± 71.52 µg.h/ml for 800 mg regimen. This is another indication for linearity of ketoconazole kinetics at the studied dosing regimens.

The values for $t_{1/2}$ were calculated as 4.24 ± 1.87 and 4 ± 0.66 h following the administration of 400 and 800 mg, respectively. No statistically significant differences were observed in $t_{1/2}$ following increase of the dose (p = 0.38). Our findings contradict the findings of 7.5 – 7.90 h reported by Huang et al, (1986) who reported a dose-dependent increase in half life with increase of the dose.

The physiological parameters Cl/F and Vd/F were estimated as 8.78 ± 6.85 and 8.79 ± 5.57 L/h ; 42.7 ± 14.67 and 49.28 ± 27.11 L for the two regimens. No statistically significant differences were observed in clearance or volume of distribution between the two regimens (P > 0.05).

4.4.4. Comparisons following the administration of the last doses of 400 and 800 mg regimens

The data obtained following the last dose was compared between the mean of four patients in the 800 mg regimen and only one patient in the 400 mg regimen. Therefore statistical comparisons were not attempted.

The peak concentrations reached following the administration of 400 mg ketoconazole were 11.56 µg/ml compared to that of 800 mg ketoconazole (18.44 ± 7.35 µg/ml). Unlike comparisons of the first doses, the values of T_{max}

following the administration of the last doses of the two dosing regimens (6.0 and 2.00 ± 0.82, respectively) were different.

The mean values of AUC_{∞} were found to be 183.07 µg.h/ml for 400 mg regimen and 264.56 ± 136.96 µg.h/ml for 800 mg regimen. While the estimated values of $AUMC_{\infty}$ were 2681.67 $\mu g.h^2$/ml and 3644.23 ± 2930.57 $\mu g.h^2$/ml.

The calculated parameters MRT, Ke and $t_{1/2}$ were found to be 14.65 and 12.42 ± 5.29 h; 0.08 and 0.11 ± 0.03 h^{-1}; 8.6 and 6.83 ± 2.15 h for 400 and 800 mg regimens, respectively. No reliable conclusions could be drawn from these observations because of the validity of the statistical test.

The Cl/F and Vd/F were estimated as 1.09 and 3.18 ± 2.48 L/h; 13.55 and 36.66 ± 40.14 L for the two regimens, respectively. There was increase in clearance and volume of distribution. Contrary to reports on the literature, the values of both Cl and Vd showed decrease following increase of the dose of ketoconazole (Huang et al, 1986).

It has been observed that there is a double peak phenomena in the plasma concentration-time profile following the administration of the last dose of 800 mg regimen. The explanation of the double peak profile is reported by several authors in the literature. The double peaks in serum concentration, seen at higher doses of ketoconazole, raise the question of enterohepatic circulation or delayed absorption as suggested by Brass et al (1982).

A random subgroup of 14 sets of samples from 12 patients showed a single peak in 9 sets (8 patients) and double peaks in 5 sets (4 patients). The possibility of demonstrating double peaks is obviously related in part to the number of samples obtained per day, but the fact that this phenomenon was frequently seen indicates that curves of concentration versus time after dose for individual patients are often irregular. Possible explanations include prolonged or irregular absorption and enterohepatic recirculation (Sugar et al. 1987)

Better absorption, nonlinear elimination, saturable first-pass metabolism, or a change in the volume of distribution of ketoconazole have also been suggested as explanations for the dose-dependent phenomena observed when doses increase from 100 to 400 mg (Daneshmend et al.1983).

Our results support the concept of a change in pharmacokinetics with chronic dosing.

4.4.5. Comparisons of steady state levels of 400 and 800 mg regimens

Plasma concentration fluctuated between minimum steady state concentrations (Cp_{ssmin}) of 2.68 ± 1.40 (CV%, 52.13%) and 4.18 ± 0.98 (CV%, 23.52%) and maximum steady state concentrations (Cp_{ssmax}) of 8.00 ± 1.65 (CV%, 20.67%) and 11.97 ± 3.24 (CV%, 27.07%) following the administration of the 400 mg and 800 mg ketoconazole regimens, respectively.

The corresponding fluctuation at steady state (F, calculated as Cp_{ssmax}/Cp_{ssmin}) was found to be 3.98 ± 3.01 for the 400 mg regimen and 2.96 ± 0.93 for the 800 mg regimen (Table 3.8).

4.5. Clinical outcome

Medical treatment before surgery is recommended to ascertain high levels of ketoconazole in the circulation to minimize the chance of the organism local spread. Treatment with ketoconazole was continued in this study until the patients are clinically, serologically and radiologically cured. Clinical improvement is judged by reduction in the size of the mass and healing of the sinuses. Cure is considered when the skin becomes normal, the mass disappeared, the sinuses had healed and the organism is eliminated from the tissue. Prognosis of both treatment groups was satisfactory. In a dose of 200 mg twice daily, no significant side effects or biochemical abnormalities are seen. Except for dry mouth, experienced by the 800 mg regimen group, all patients were free of side effects. Liver function tests are carried out at baseline and at three months after treatment and no significant differences were observed.

It has been mentioned earlier that surgical reduction of a bulky swelling without affecting the function of an affected bony lesion will certainly reduce the length of treatment. Both treatment groups showed reduction in bulky swelling and the lesions were well encapsulated. This allowed avoidance of aggressive surgical excision or amputation.

Only one patient in the 800 mg group experienced recurrence. Recurrence is more common after an incomplete course of medical treatment and there is a good chance for the organism to develop drug resistance.

Conclusion

It has been reported that the half-life of ketoconazole seems to be dose-dependent, increasing with increasing dose and after repeated dosing (Huang et al, (1986), Gascoigne et al, (1981). The slower elimination phase is also dose-dependent. Thus, long-term administration of ketoconazole can result in drug accumulation in humans (Heel et al, 1982) in rats and dogs (Gascoigne et al, 1981), and in mice (Whitehouse et al, 1990). The increasing dose-dependent half-life during long-term treatment in humans suggests autoinhibition of metabolism (Heel et al, 1982). Furthermore, ketoconazole displays nonlinear kinetics in both the volume of distribution and clearance as the dose is escalated (Huang et al, 1986). These observations correlate with the first-pass elimination of ketoconazole, with transient saturation of the drug-metabolizing capacity of the liver at higher dosages (Heel et al, 1982, Huang et al, 1986 and Gascoigne et al, 1981). Also, ketoconazole has been purported to be extensively metabolized to a large number of metabolites, with hepatic microsomal enzymes playing the major role in the biotransformation reactions (Gascoigne et al, 1981 and, Daneshmend and Warnock, 1988). The metabolic pathways suggested in ketoconazole's biotransformation include oxidation, cleavage, degradation, and scission of the imidazole and piperazine rings, oxidative O-dealkylation, and aromatic hydroxylation (Heel et al, 1982, Gascoigne et al, 1981 and Medoff and Kobayashi, 1980). N-deacetyl ketoconazole appears to be the major metabolite reported in mice (Whitehouse et al, 1990). The accumulation of ketoconazole in mouse liver was minimal, whereas the hepatic levels of N-deacetyl ketoconazole were significant (Whitehouse et al, 1994).

The binding of ketoconazole in liver and adrenals is significant as shown by the liver to plasma partition coefficient of about 22 at a steady-state plasma concentration of 0.015 mg/L. Since this binding phenomenon is concentration dependent, as the tissues sites are saturating at elevated

concentrations, the volume of distribution of the drug decreases [Matthew et al. 1993]

The hepatic clearance of ketoconazole is saturable such that AUC increases disproportionally with increasing dose [Matthew et al. 1993, Daneshmend et al.1984]. It was found that any dose resulting in concentrations above 0.5 mg/L would be anticipated to show mark nonlinearity [Matthew et al. 1993]

Our findings are in good agreement with the above mentioned findings. They suggest the validity of the use of both dosing regimens of ketoconazole for the treatment of eumycetoma. However, the use of 800 mg dosing regimen is recommended to be use with great caution and only if necessary provided that continuous monitoring of the liver and kidney functions is grunted. Since there is a risk of dose-dependent pharmacokinetics which results in non-linear kinetics in clearance and volume of distribution. In addition to the possibility of autoinhibition of ketoconazole metabolism following increase of the dose and repeated dose administration. All this is expected to cause accumulation of ketoconazole in the body and might lead to toxicity.

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