CHAPTER 1

1 INTRODUCTION

1.1 TUBERCULOSIS AND HIV CO-INFECTION

"Ebb and Flow: The Failed Conquest"
(Barry Bloom, 1994)

Tuberculosis, a disease of great antiquity, is still a global emergency today (WHO, 1994). While the 1950’s documented a steady decline in the incidence of tuberculosis, by the 1980’s this trend had changed dramatically. The disease reemerged with a vengeance and the conquest, which seemed imminent, now remains an illusion. A well recognized and often stated fact is that tuberculosis (TB) is now the world’s foremost cause of death from a single infectious agent, having infected up-to one third of the world’s population (Raviglione and Nunn, 1997).

TB is defined as an infectious disease caused by mycobacterium tuberculosis. “Characteristic features include a generally prolonged latency period between infection and overt disease, prominent pulmonary disease and a granulomatous response associated with intense tissue inflammation and damage”\(^1\)

The diagnosis, treatment and prevention of tuberculosis is generally well known, yet it is killing more individuals today (a global estimate of 3 million deaths per annum) than when Robert Koch discovered the mycobacterium more than a century ago (WHO, 1996).

There are many factors implicated in the growing resurgence of the disease. The consequences of world population growth, poverty, and neglect of TB control programs are but some of the causes. However the impact of the Human Immuno-deficiency Virus (HIV) is the single most significant factor (WHO, 1994; 1996).

HIV causes a persistent infection in human hosts that ultimately results in AIDS (Acquired Immune Deficiency Syndrome), which represents the end of the clinical spectrum. HIV-1 strains can be further separated into subtypes A-J which belong to the main group M and a minor group O. The virus has a particular tropism for CD4 cells (T-helper cells: main 'conductor' of the immune system) in general and activated memory cells in particular causing their depletion thus decreasing the ability of cell mediated immune response. Individual infection is characterized by long and highly variable incubation period between the moment of infection and the development of AIDS (can last from 1 to more than 15 years).

The Hippocratic description of tuberculosis as 'phthisis' a Greek term for 'wasting away' (Bloom ed., 1994) aptly portrays the current clinical scenario of TB/HIV co-infection.

1.2 THE EFFECTS OF HIV ON TUBERCULOSIS

1.2.1 GLOBALLY AND IN AFRICA

Co-infection with HIV greatly influences the clinical course of tuberculosis. This has been evident in the past decade during which an emergence of the changing clinical pattern has been witnessed (Chintu and Zumla, 1997; Huebner and Castro, 1995; Whalen et al., 1995). Human Immuno-deficiency Virus (HIV) is currently the most potent known cause of an infected person developing TB (Elliot et al., 1990; Lucas and Nelson, 1994).
HIV-related tuberculosis may result from either primary infection of a previously uninfected person, endogenous reactivation of latent disease or an exogenous re-infection (Chretien, 1990). In high prevalence areas such as Africa, reactivation is the commonest cause of the disease (Rouillon et al., 1976). In 1996, the number of ‘dually’ infected patients in the world was estimated to be 6,000,000. From this figure it was postulated that 480,000 of the cases were of overt tuberculosis, of which 300,000 were in Africa (Dolin et al., 1994).

Lienhardt and Rodrigues (1997), associated the following effects with HIV:

- an increase in the chances of an infected person developing overt disease
- a considerable reduction in the time interval between infection and the manifestations of such disease
- the clinical features of the disease, notably in the profoundly immuno-suppressed are modified
- increases the rate of transmission of infection in the general population as a result of enhanced number of source cases

TB/HIV co-infection, the ‘cursed duet’, has fueled the spread of TB throughout Sub-Saharan Africa with a tripling in the number of TB notifications in the past decade (WHO, 1994). At present 10% of all cases of tuberculosis worldwide and between 30 to 70% of those in Africa are HIV related (WHO, 1997). By the year 2000 the number of overt tuberculosis cases worldwide will be nearly 1.4 million and 600,000 of these will be in Africa (Dolin et al., 1994). Worst case scenario for Africa beyond the year 2000 is that 1 in 50 of the total population and 1 in 25 of the at-risk population, could develop tuberculosis each year (Schulzer et al., 1992).

A detailed study in the USA showed a higher mortality in HIV co-infected patients with TB than without; matched by T-helper cell lymphocyte (CD4) counts per degree of immunodeficiency (Chaison et al., 1987). The excess mortality in TB/HIV co-infected patients during and after treatment is partly due to the TB itself and the rest due to other HIV related disorders. HIV is undoubtedly adverse for TB, but there is growing evidence that the reverse is also true. Recent documentation suggests that active tuberculosis boosts retroviral (HIV) replication by
unknown mechanisms (Whallen et al., 1997; Golleti et al., 1996). These observations indicate that an additional benefit of comprehensive treatment of tuberculosis in HIV/TB patients may be the prevention of tuberculosis induced HIV progression.

1.2.2 THE ZAMBIAN BURDEN

Zambia, with a reported population of 7.8 million (1990 national census) and current growth rate of 2.7% per annum, has experienced urbanization on a large scale in the previous two decades (over 45% live in urban areas). This has lead to a scenario similar to Europe in the 1800’s, with social amenities (i.e. housing, water supply and sanitation) being over stretched. The poor economic performance accompanied by the resultant high unemployment rate has further compounded the issue (ZDHS, 1996). As a consequence the AIDS pandemic has reached tragic proportions leading to the virtual breakdown of the national TB control program (unpublished personal observations). Recent research has shown a high prevalence of HIV in TB patients and that it is one of the commonest causes of admission in adults and children at the University Teaching Hospital (UTH) in Lusaka, Zambia (Elliot A, Luo N, et al., 1990). Over 60% of children and 70% of the adults with tuberculosis are co-infected with HIV (Chintu et al., 1997). The annual incidence of TB in Zambia has risen from 100/100,000 between 1975-1985 to 350/100,000 in 1994 (Ministry of Health statistics: latest figures are estimated to be closer to 450/100,000). Similar increments have been noted in other countries of East, Central and Southern Africa (WHO, 1996).

Two year follow-up studies (by Elliot et al., 1995) of HIV infected patients with TB showed alarmingly significant mortality, treatment failure and relapse rates in Zambia. The crude 2 year mortality rate ratio for HIV positive as compared with HIV negative patients was 5.00. The recurrence rate after completion of anti-tuberculosis therapy was found to be four times higher in HIV-infected patients (22/100 person years) as compared to HIV negative patients (Elliot et al., 1995). The official Life expectancy at birth in Males was 46.1 and in Females was 47.6 years (1990 census). However with the current ‘cursed duet’ these already appalling figures are expected to dramatically worsen in the next census!
New Cases of Tuberculosis in Zambia

Notification Rate /100,000/year 1974-1996

Source: MoH AIDS/STD/TB Programme

(1996 data provisional)
Incidence of Tuberculosis, Zambia 1996

rates per 100,000 population

Notification Rate

- 100 to 249
- 250 to 349
- 350 to 449
- 450 to 549
- 550 to 849
With the onset of the AIDS pandemic and the subsequent increase in active TB cases, the treatment of TB has taken on an even greater significance. The World Bank recognizes good anti-TB treatment as one of the most cost-effective health interventions. The WHO’s ‘strategy and framework for effective TB control’ in response to the global emergency has incorporated the Directly Observed Treatment Short course (‘DOTS’) regimen with encouraging results in areas where fully implemented (WHO, 1998). The DOTS course (recently implemented in Zambia) comprises the following four drugs namely: isoniazid, rifampicin, pyrazinamide and ethambutol. Isoniazid and rifampicin are the most effective bactericidal drugs; rifampicin and pyrazinamide are the main sterilizing drugs as they kill different sub-populations of semi-dormant organisms. Finally rifampicin and isoniazid are the most effective in preventing the emergence of resistance to other drugs. An important point in the use of anti-tuberculous drugs is that they have to be used in combination otherwise mycobacterial resistance develops rapidly!

The individual properties of the first-line anti-tuberculous drugs are amplified below.

1.3.1 ISONIAZID

Isoniazid (isonicotinic acid hydrazide) is still considered to be the most important drug for tuberculosis chemotherapy.

It is a weak base that is soluble in water, ethanol and methanol. The stability of the drug in frozen plasma samples is contentious; therefore it would be wise to deproteinise samples within 6 hours of collection or extract the drug as soon as possible. Isoniazid (molecular wt. 137.1) is available clinically as tablets or capsules or as combined formulations with Rifampicin, Ethambutol or Pyrazinamide. Parenteral preparations of 50mg in 2ml are also available. Commonly used dose is 5 mg/kg with a maximum dose of 300 mg (per oral or parenteral).

Isoniazid is a strong bactericidal drug with few side effects. The mechanism of action is unknown but is thought to act by inhibiting the biosynthesis of mycolic acid of the cell wall.
Table 1(a).

Pharmacokinetic properties and MICs of anti-mycobacterial (first-line) drugs

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg)</th>
<th>Fraction absorbed (%)</th>
<th>Peak (mg/L)</th>
<th>Half-life</th>
<th>Protein binding</th>
<th>MIC (mg/L) for M. tuberculosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoniazid</td>
<td>300</td>
<td>Well absorbed*</td>
<td>5 (slow-acetylators)</td>
<td>3 hours</td>
<td>0-20</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>5mg/kg</td>
<td></td>
<td>4 (rapid-acetylators)</td>
<td>1.3 hours ↑ in cirrhosis, neonates, uremia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rifampicin</td>
<td>600</td>
<td>Well absorbed*</td>
<td>10</td>
<td>3 hours ↑ hepatitis, cirrhosis, uremia.</td>
<td>85</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>10mg/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethambutol</td>
<td>1200</td>
<td>70-80</td>
<td>3</td>
<td>4 hours ↑ uremia</td>
<td>20</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>15mg/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyrazinamide</td>
<td>2000</td>
<td>Well absorbed*</td>
<td>40</td>
<td>9 hours ↑ cirrhosis</td>
<td>0-40</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>15-30 mg/kg</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

* Absorption is almost 100% but food and other medication can decrease this. In addition hepatic 1" pass effect can also be a factor though the metabolites produced can still be active.

It is well absorbed (oral and intramuscular) and widely distributed throughout the body water, with peak plasma concentrations of 3 to 5 ug/ml one to two hours after the oral dose. Isoniazid readily penetrates into cell body fluids; concentrations in the cerebrospinal fluid are similar to
those in plasma (Holdiness, 1985). Significant levels are also attained in caseous material.

75% to 95% of the isoniazid dose is excreted in urine within 24 hours, mostly as metabolites (as a result of enzymatic acetylation and hydrolysis). Human populations show considerable genetic variation with regard to the rate of acetylation (Evans et al., 1960). Subjects are characterised as either slow or fast acetylators (High acetyl transferase activity, “fast acetylators” is inherited as an autosomal dominant trait). Rate of acetylation significantly alters concentration of drug in plasma and it’s half-life in circulation. Mean half-life of fast acetylators is approximately 70 minutes, whereas 2 to 5 hours is characteristic of slow acetylators. Hepatic insufficiency may also prolong the half-life.

Adverse reactions are unusual. Peripheral neuropathy is the most common side effect if concurrent Pyridoxine (prophylaxis dose = 10 mg) is not given. It occurs as result of isoniazid’s inhibition of the action of coenzymes produced from pyridoxine and is related to plasma levels of the drug. It is more frequent in slow acetylators and in individuals with diabetes, HIV, poor nutrition or anaemia. Hypersensitivity may result in fever, hepatitis and various skin eruptions (morbilliform – urticarial). Hepatitis incidence increases with age (> 30 years) and worsens if drug is continued after symptoms of hepatic dysfunction have appeared. It is recommended that the drug be discontinued if an elevation of greater than 5 fold of serum aspartate amino transferase is noted (Byrd et al., 1979). Rare side effects are convulsions, toxic encephalopathy, euphoria, florid psychosis, hemolytic and aplastic anaemia, methaemoglobinemia, arthralgia, gynaecomastia and lipid reactions.

1.3.2 RIFAMPICIN

Rifampicin (a rifamycin) is a key component of any antituberculosis treatment. It is a semi-synthetic derivative of rifamycin B and is freely soluble in organic solvents as well as water at an acidic pH. Rifampicin (molecular weight- 822.9) is available clinically as capsules, tablets or syrup in addition to the combined preparations mentioned above.
The dose of rifampicin for the treatment of tuberculosis in adults is 600mg (450mg if weight < 50kg). It is preferable to take the drug on an empty stomach, as food is known to impair the absorption (either 1 hour before or 2 hours after the meals). The drug is bactericidal for both intracellular and extracellular organisms. It acts by inhibiting the enzyme DNA dependent RNA polymerase of the mycobacterium, forming a stable drug-enzyme complex; thus leading to suppression of initiation of chain formation in RNA synthesis. Rifampicin should never be used alone in tuberculosis due to the rapid development of resistance (one per 10 million tubercle bacilli are resistant to it). In addition to the mycobacteria, it is also active against most gram positive as well as gram negative organisms.

The oral ingestion of 600mg will produce peak plasma concentrations of about 7 ug/ml in 2 to 4 hours. Aspirin may impair absorption (Radner, 1973) to such an extent that adequate plasma levels may not be reached. After absorption, rifampicin is rapidly eliminated in bile. Enterohepatic circulation ensues and the drug is progressively deacetylated consequently being completed within 6 hours. However the metabolite retains full antibacterial activity, though intestinal reabsorption is reduced. Rifampicin is a potent inducer of hepatic microsomal oxidases and is extensively metabolised by the liver. Therefore repeated dosages will induce it's own metabolism so reducing its elimination half life from 3.5 hours to 2 hours during the first 14 days of treatment. After oral administration 30% of the drug is excreted in urine and 60 – 65% in faeces.

Rifampicin is widely distributed throughout the body and is present in effective concentrations in many organs and fluids, but passes poorly into the cerebrospinal fluid, where concentrations are only 8% or less of the concomitant serum concentrations. Higher levels are found when the meninges remain inflammed (Ellard et al., 1993).

Rifampicin is generally well tolerated but imparts a red-orange colour to the body fluids i.e. tears, saliva, urine, faeces, etc. (Furesz, 1970). Cutaneous reactions in the form of mild flushing and pruritis occur infrequently. Hepatitis is extremely uncommon unless patient has history of liver disease or alcoholism. Other rare syndromes may occur in patients taking the drug intermittently, namely:

- ‘Influenza’ syndrome: shivering, malaise, headache and bone pains.
• Thrombocytopenia and purpura: platelets drop to very low levels and haemorrhage occurs. Treatment should be stopped immediately.
• Respiratory and shock syndrome: dyspnoea, wheeze, hypotension and collapse. Corticosteroid therapy may be required.
• Acute haemolytic anaemia and renal failure. Rifampicin should not be reintroduced again if shock syndrome, acute haemolytic anaemia or acute renal failure has occurred.

The induction of hepatic microsomal enzymes by rifampicin causes a significant reduction in serum half-life and clinical efficiency of a number of drugs. These include morphine, methadone, phenobarbitone, digoxin, corticosteroids, coumarin anticoagulants and oral contraceptives.

1.3.3 ETHAMBUTOL

Ethambutol is a water-soluble and heat stable compound. It is strongly basic with a molecular weight of 277.2. The drug is tuberculostatic and is thought to act by inhibiting the incorporation of mycolic acid into the mycobacterial cell wall (Takayama et al., 1979). Bacterial resistance develops if given in absence of any other drug.

Clinically the drug is available for oral use as powder or tablets containing hydrochloride or as a combined tablet with isoniazid. Usual dose is 15 mg/kg though some physicians use 25 mg/kg for the first 60 days then reduce to 15 mg/kg per day. It is mainly used in combination therapy to prevent emergence of drug resistance.

About 75% to 80% of an orally administered drug is absorbed from the gastrointestinal tract. Peak plasma concentrations are reached 2 to 4 hours after the drug is taken and is proportional to the initial dose (25 mg/kg will produce a plasma concentration of 2 to 5 μg/ml). The drug is widely distributed though penetrates the blood-brain barrier poorly, though penetration is improved when the meninges are inflammed. Within 24 hours three quarters of the drug ingested is excreted unchanged in urine by tubular secretion as well as glomerular filtration. Up to 15% is excreted in the form of inactive metabolites, dialdehyde and dicarboxylic acid. Despite the dependency on renal excretion, only a
modest increase in elimination half-life has been reported in patients with renal impairment. However a recent review has concluded that ethambutol should not be recommended for treatment of patients with renal failure because of the risk of overdose (Ellard, 1993).

Ethambutol is well tolerated but should not be used to treat children, in whom retrobulbar neuritis is difficult to diagnose. This neuritis causes a progressive loss of visual acuity and loss of ability to differentiate red from green. It is dose dependent and is reversible if the drug is removed from the regimen. Drug must be stopped immediately failing eyesight is noticed as if it is continued than the patient can become permanently blind.

Ethambutol will increase serum urate levels due to decreased renal excretion (Postlethwaite et al., 1972) but this is not normally clinically significant. Other rare minor side effects are rash, drug fever, arthralgia, mental confusion, and peripheral neuritis.

1.3.4 PYRAZINAMIDE

Pyrazinamide is a bactericidal drug, only active against intracellular dividing forms of Mycobacterium tuberculosis at an acidic pH. It is a synthetic pyrazine analogue of nicotinamide (molecular weight of 123.1) which is weakly basic and readily soluble in water and organic solvents. It's mechanism of action is unknown. As with other anti-tuberculosis drugs, resistance develops rapidly if used alone.

Despite the fact that it’s MIC is high and therapeutic doses only achieve a two-fold increase (above the MIC) in serum levels, pyrazinamide has a remarkable sterilizing activity when used in multi-drug regimens (main effect in first two to three months). It is available clinically as oral tablets as well as combination formulations with isoniazid and rifampicin (rifater®). The daily dose for adults is 15 to 30 mg/kg in three to four equally spaced doses. However, a single daily dose has also been found to be safe and effective.

Pyrazinamide is rapidly absorbed from the gastrointestinal tract and distributed throughout the body water. It has good meningeal penetration (CSF levels are similar to concomitant plasma levels), therefore it is
particularly useful for tuberculous meningitis. Oral administration of 1g will produce plasma concentration of 45 ug/ml at 2 hours and 10 ug/ml at 15 hours. Binding to plasma protein is not thought to occur, though a recent study by Woo et al, (1996) placed it at 40%. The drug is mainly excreted by renal glomerular filtration; urinary concentrations of 50 to 100 mg/ml are attained for several hours after a single dose. Pyrazinamide is extensively inactivated by hydroxylation to pyrazinoic acid and 5-hydroxy pyrazinoic acid (the major excretory products).

Untoward effects are normally mild, and include cutaneous hypersensitivity, anorexia, nausea and arthralgia. The most serious side effect is injury to the liver (this is dose dependent). A 40 to 50 mg/kg dose will produce clinical evidence of hepatitis in 15% of patients, with jaundice in 2 to 3 % and death due to hepatic necrosis in rare cases. Elevation of the plasma levels of the liver enzymes is the earliest abnormality. Regimens currently employed (15 to 30 mg/kg per day) are much safer (Girling, 1978). The drug should not be given to individuals with any degree of hepatic dysfunction unless absolutely unavoidable. As the metabolite, pyrazinoic acid inhibits the excretion of urate, acute episodes of gout have occurred. This can be treated with analgesics.

1.3.5 STREPTOMYCIN

It is an aminoglycoside, derived from *Streptomyces griseus*, which was the first clinically effective drug against tuberculosis. The toxicity (due to the initial high doses) and development of resistant bacilli limited its usefulness. However the use of lower dosages and the discovery of other antimycobacterial drugs (leading to multi-drug regimens) enabled a far more effective treatment of tuberculosis. At present it is the least used of the “first-line” agents and is reserved for more serious forms of tuberculosis e.g. disseminated disease or meningitis. The in vivo activity of streptomycin is essentially suppressive. This may be related to the fact that the drug does not readily enter living cells thus cannot kill intracellular microbes.

Dose is 15 mg/kg (intramuscular) per day. Therapy is usually discontinued after 2 to 3 months or sooner if cultures become negative, as side-effects increase after accumulative dose of 100g.
<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg)</th>
<th>Fraction absorbed (%)</th>
<th>t½ (hours)</th>
<th>Common side-effects</th>
<th>MIC (mg/L) for Myco. Tuberculosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ofloxacin</td>
<td>300-600mg</td>
<td>100</td>
<td>5.7</td>
<td>Nausea, dyspepsia, diarrhoea, rash (rarely steven johnson syndrome), pruritis, dizziness insomma. Less common- liver &amp; hepatic dysfunction, hallucinations, convulsions, hypersensitivity reactions, blood disorders.</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacín</td>
<td>500-750mg</td>
<td>60</td>
<td>4.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethionamide</td>
<td>15-20mg/kg (increase dose slowly)</td>
<td>Take with meals to avoid gastric irritation</td>
<td>2</td>
<td>GIT upset, nausea, vomiting, metallic taste, depression postural hypotension, asthenia, and hepatitis. Convulsions &amp; peripheral neuropathy are rare.</td>
<td>2.5</td>
</tr>
<tr>
<td>PAS</td>
<td>10-20g/day</td>
<td>Readily absorbed. Take with meals</td>
<td>1</td>
<td>Anorexia, nausea, gastritis, diarrhoea, hypersensitivity reaction, fever, arthralgia, leukopenia, agranulocytosis</td>
<td>1</td>
</tr>
<tr>
<td>Cycloserine</td>
<td>250-500mg</td>
<td>-----</td>
<td>1</td>
<td>CNS symptoms: somnolence, headache, tremor, confusion, seizures</td>
<td>5-20</td>
</tr>
<tr>
<td>Amikacin</td>
<td>15mg/kg</td>
<td>-----</td>
<td>2.3</td>
<td>Oto- &amp; nephrotoxicity</td>
<td>4</td>
</tr>
<tr>
<td>Capreomycin</td>
<td>15-30mg</td>
<td>-----</td>
<td>5.2</td>
<td>Hearing loss, transient proteinuria, leukocytosis. Severe renal failure is rare</td>
<td></td>
</tr>
<tr>
<td>Kanamycin</td>
<td>1g</td>
<td>-----</td>
<td>2.1</td>
<td>Neuromuscular paralysis, respiratory depression, agranulocytosis, anaphylaxis, nephrotoxicity, rash</td>
<td>10</td>
</tr>
</tbody>
</table>
As aminoglycosides are highly polar cations, streptomycin is poorly absorbed from the gastrointestinal tract (less than 1%). It is rapidly absorbed from intramuscular sites with peak plasma concentration reached after 30 to 90 minutes. Because of the same polar nature, streptomycin is excluded from mast cells, central nervous system and the eye. Inflammation increases penetration into pleural and pericardial cavities. Concentration in CSF is less than 10% of plasma levels in the absence of inflammation and may approach 25% in patients with meningitis (Strausbaugh et al., 1977). It is excreted almost entirely by glomerular filtration, thus plasma drug concentrations should be measured in patients with impaired renal function (the dose may have to be adjusted, depending on the degree of impairment). The concurrent use of furosemide may potentiate the nephrotoxicity of streptomycin (Brummet, 1983).

About 8.2% will manifest with adverse reaction; 50% of which will involve auditory and vestibular function. Other manifestations include rash (in 2%) and fever (in 1.4%). Streptomycin predominantly affects the vestibular apparatus and only rarely causes ototoxicity. Damage to the vestibular apparatus presents with giddiness, and if acute may also have vomiting. Unsteadiness is more marked in the dark and nystagmus may be present damage to the nerve may be permanent if the drug is not withdrawn but if stopped immediately than symptoms usually clear over a week (Wilson et al., 1984). It can cause hearing impairment in foetuses therefore streptomycin should be avoided in pregnancy. The intramuscular injections may cause anaphylaxis

1.4 EFFECTS OF HIV ON ANTI-TUBERCULOUS DRUGS

With the challenging problem posed by the AIDS Pandemic and subsequent increase in active tuberculosis cases, the treatment of tuberculosis has taken on an even greater significance. The World Bank recognizes good anti-TB treatment as one of the most cost-effective health interventions (Maher D, 1996). The World Health Organization’s ‘strategy and framework for effective TB control’ has responded by incorporating the “Directly Observed Treatment Short course” regimen (DOTS). This ensures compliance and has met with
encouraging results in some quarters where fully implemented (WHO TB program, 1996).

The current anti-tuberculous treatment regimen produces lasting cure with low recurrence rates in cases of tuberculosis patients without HIV. This treatment appears to be as effective bacteriologically in HIV positive patients with a few exceptions (Sunderam et al., 1987; Scott et al., 1997). However follow up studies of patients co-infected with TB and HIV showed significant mortality both during and after treatment, as well as higher treatment failure and relapse rates. Such patients were found to be four times as likely to die compared to HIV negative patients (Elliot et al., 1995; J van de Broek, 1998).

The high mortality, relapse and treatment failure rates in the HIV infected individuals could be attributed to poor immune function, poor compliance, poor quality drugs, drug resistance, poor gut absorption of anti-TB medication, or a combination of the above (Berning SE et al., 1992). As patients with HIV infection are more likely to relapse or acquire a new infection on completing treatment, preventive anti-TB therapy would be desirable (Scott et al, 1997). However the risk of developing drug resistance as well as the side effects has led to intense debates (Angell, 1997; Msamanga and Fawzi, 1997).

It is well recognized that the gastrointestinal tract is a major target organ in AIDS (Gottlieb et al., 1983). Progressive weight loss, chronic diarrhoea and malabsorption are among the most striking features in HIV. In parts of Africa this is so prominent that AIDS is referred to as ‘slim disease’ (Serwadda et al., 1985).

Recent case reports have suggested that malabsorption as well as delayed absorption was a possible cause of the TB recrudescence and the development of drug-resistance observed in HIV positive patients (Peloquin et al., 1993; Gordon et al., 1993). This could be attributed to HIV associated enteropathy which is either occult or overt (Bartlett et al., 1992). This could lead to the potential possibility of ‘monotherapy’ thus increased incidence of multi-drug resistance. Large-scale studies have shown that approximately 30-60% of patients with AIDS complain of chronic or intermittent diarrhoea (Dupont HL, Chintu C, Zumla A, 1998). Many have intermittent symptoms and the small bowel, which is the
predominant site for the absorption of the oral anti-tuberculous drugs, is also the commonest site of the pathological changes (Carlson S, 1994).

A myriad of opportunistic infections such as *Mycobacteria, Microsporidia, Cryptosporidia, Candida species, Cytomegalovirus* and enteroviruses frequently target the intestinal tract. The most common opportunistic pathogens responsible for chronic diarrhoea (defined by intermittent or continuous loose motions for over a month) are: *Cryptosporidium, Microsporidium, Mycobacterium avium*, and *Cytomegalovirus*. Each accounted for 15-40% of the cases in a large number of series. Studies in Lusaka, Zambia have shown protozoan organisms such as *Cryptosporidium, Microsporidium, and Isospora belli* as the three most common causes of HIV-related persistent diarrhoea. Kelly et al found *Cryptosporidium* to be the commonest organism in 1996 whereas Zulu et al found *Isospora belli* to be more common in the same year. These parasites have similar histopathological findings and in addition have documented D-xylose malabsorption.

Xylose is a sugar commonly used to measure intestinal integrity. The **D-Xylose test** is carried out by giving 25g of D-Xylose to a well-hydrated subject. A blood level greater than or equal to 30 mg/dL one hour after ingesting xylose may indicate normal absorption. A low level indicates poor mucosal absorption or contamination of the jejunum by bacteria that metabolize the sugar before it can be absorbed.

Several studies have shown the effects of this enteropathy on the absorption of anti-retroviral and other medication including antituberculous drugs (Berning et al., 1992; Peloquin et al., 1993; McNab et al., 1993). Chronic diarrhoea is a common feature of HIV infection and is present in 30% of the tuberculosis patients in Lusaka, Zambia (Dupont et al., 1998).

After the resurgence of tuberculosis due to the ‘cursed duet’ the world is now faced with a potential ‘third epidemic’ of multidrug resistance disease (Neville et al., 1994). The documented suboptimal serum levels of available drugs has led to the belief that there will be widespread emergence of strains resistant to one or more of the first-line drugs (Peloquin et al., 1996) though statistically significant data has yet to be documented (Anastasis et al., 1997). In sub-Saharan Africa, the incidence
of multi-drug resistance per 100,000 of population in the year 2000 could range from 2.3 to 32 (Carpels et al., 1995).

The shortening of life, despite adequate anti-tuberculosis chemotherapy, is a serious cause for concern and emphasis the need for more intensive research into ways of preventing the development of overt disease in co-infected persons.

1.5 JUSTIFICATION

Despite the magnitude of the current HIV-Tuberculosis pandemic, there have not been any previous scientific studies on the pharmacokinetics of anti-TB drugs in HIV infected Zambian adults. This has largely been due to the difficulties with drug assaying (Peloquin, 1992) and the cost implications. It was found to be more economical to monitor the clinical end-point (i.e. cure) than the serum levels. However with the increased morbidity and mortality as well as the increased incidence of multi-drug resistant TB associated with the co-infection, expense should no longer be an issue!

Many of the assay methods for the antimycobacterial drugs are neither simple nor routine. Requests for measurements of any of the antimycobacterial drugs need to be assessed, to consider if the value of the information obtained is worth the cost of the procedure. Many of the drugs used are bactericidal and their therapeutic efficacy is not directly related to their plasma concentration or to the time for which blood levels exceed their MIC. Therefore findings of abnormally high or low serum drug levels is not necessarily the true reflection of activity of the drug at site of the disease process. Incidences of poor absorption, and thus inadequate serum concentrations are exceedingly rare, at least in non-AIDS patients, and by far the commonest reason for treatment failure is non-compliance.

More sophisticated methods, such as High Performance Liquid Chromatography (HPLC), fluorimetric, spectrophotometric, and immunological assays, have been developed for measuring serum
concentrations and are useful for research investigations of pharmacokinetics, measurements where samples are low, or in determination of CSF levels. In-patients with clinically significant hepatic impairment, monitoring serum levels of isoniazid, rifampicin and pyrazinamide could be helpful though exact relation between blood levels and liver damage is unclear. AIDS patients would benefit from closer monitoring as cases of abnormal pharmacokinetics have been documented (see below), particularly impaired oral absorption.

Case reports and studies from the developed world (Zorza, 1993; McNab, 1993; Peloquin, 1996) suggested that TB recrudescence or development of drug resistance in HIV positive patients could be due to HIV associated enteropathy, though other variables could not be excluded. In addition one of the reports noted that delayed absorption occurs (Peloquin, 1996). There is a high prevalence of occult and clinically apparent enteropathy in HIV infected individuals worldwide. Studies on HIV in the gut in Lusaka, Zambia and other Central African countries have shown even asymptomatic HIV infected individuals with normal CD4 counts have evidence of HIV enteropathy (Dupont et al., 1998; Kelly et al., 1995).

Nearly a quarter of HIV infected adults and children at the UTH with TB have been found to have diarrhoea (Chintu C, Dupont HL, 1998). Similar findings were noted in other parts of Africa (Lucas et al., 1994; Perriens et al., 1995). Several studies in Europe have shown adverse effects of the enteropathy on the absorption of anti-retroviral drugs. A study by Peloquin et al. (1996) documented that low antituberculous drug serum concentrations occurred frequently during treatment of patients with AIDS. However Chaudry et al. (1996) could find no significant differences in the pharmacokinetics of patients with AIDS and those without in Kenya.

While the WHO’s DOTS regimen appears to ensure compliance, therapeutic monitoring is not widely practiced because of the difficulties in assaying of drugs, the cost and the questions raised concerning the actual pharmacological profile at the site of disease process.

The above points raise important questions concerning the bioavailability of anti-TB drugs and whether the use of regular therapeutic drug
monitoring should be adopted in view of the possibility of development of MDR-TB. The pharmacokinetics of anti-tuberculous drugs in HIV infected Zambian patients’ remains to be determined.

1.6 AIMS AND OBJECTIVES

This study attempts to define whether HIV infected Zambian patients, especially those with associated enteropathy, can achieve therapeutic levels of anti-TB drugs.

The specific objectives of the study are:

➢ To assess whether HIV infected individuals with TB can achieve therapeutic levels of the anti-TB drugs in their plasma after oral administration of a single dose.

➢ To define the steady-state pharmacokinetics of antituberculosis drugs in adult Zambian patients.

➢ To determine if there are any significant differences in the steady-state plasma levels of anti-TB drugs between:

   a) HIV infected TB patients with diarrhea

   b) HIV infected TB patients without diarrhea

   c) HIV-negative TB patients without diarrhea.
CHAPTER 2

2 MATERIALS AND METHODS

"Though this be madness, yet there is method in it"
(Hamlet; scene II, act 2)

2.1 DESIGN

The study was designed as a cross-sectional, comparative study between HIV positive PTB patients with and without diarrhoea and HIV negative patients serving as control. Standard doses of anti-tuberculous treatment were administered for at least a week to the subjects' to allow steady state of Rifampicin to be reached prior to the actual samples' collection. The drugs to be analyzed had included Ethambutol but delay in validating a method for analysis precluded its inclusion in the study. Ethical approval to conduct the study had been obtained from the Research and Ethics Committee of the University of Zambia.

2.2 SETTING

The recruitment of patients, baseline investigations and specimen collection had taken place at the University Teaching Hospital in Lusaka, Zambia.

The drug assays of the collected samples was done by utilizing the laboratory facilities of the University College London Medical School in England as similar facilities or the expertise were not available locally.
2.3 SUBJECTS

Potential patients with a clinical diagnosis of tuberculosis who had either been admitted to the medical wards at the University Teaching Hospital (bed capacity of 1,400) or had presented to the chest clinic at the same institution (catchment area covered includes a population just over 1.8 million) were identified (ZDHS, 1996).

They were required to meet all the following criteria below for admission into the project:

- Adults aged between 16 and 60 years.
- Should have received antimycobacterial drugs for at least a week (to overcome the inducing effects of Rifampicin).
- No co-existing chronic medical illness (especially disseminated Karposi’s sarcoma) apart from HIV disease and diarrhea.
- No significant hepatic or renal dysfunction (levels of transaminases and total bilirubin < 3 times normal and no abnormal levels of urea and serum creatinine i.e. levels > 140umol/L).
- Not be pregnant or breast-feeding.
- No oral thrush or symptoms of dysphagia (to rule out candidiasis).
- Hemoglobin was to be greater than 10g/L.
- On being informed verbally, as well as in writing about the project they had to be willing to undergo admission and the sampling procedure.

Patients fulfilling the admission criteria were subsequently recruited. A total of 60 patients comprising the following three groups were enlisted:

a) HIV-positive patients with pulmonary TB and no diarrhoea

b) HIV-positive patients with pulmonary TB and chronic diarrhoea (defined by production of 3 or more unformed stools per day for at least three days/ week for the past one month.
c) Isoniazid- 300mg in persons below 50kg and 400mg in persons above.

The drugs used were the same as the patients' regular supplies (i.e. manufactured by Pharmamed®).

5 to 10ml of venous blood was collected and stored in preservative-free heparinised vials at the following points:

- Baseline before administration of drugs.
- One hour after administration of drugs.
- Two, four, six, eight and twenty-four after administration of drugs.

The patients were then catered for with a delayed breakfast (2 hours after ingestion of the medication). This was to prevent food from interfering with the pharmacokinetics of the drugs. Subsequent meal times were followed normally.

The samples obtained were immediately centrifuged at 2000rpm for 15 minutes and the plasma decanted into freeze tubes, 5ul of Sodium- Azide added and the solution then stored at minus 70°C. Plasma samples obtained were labeled P0, P1, P2, P4, P6, P8, and P24. In addition each tube had the patients study number affixed to it.

The rational for these collection times was due to the fact that the varied half-lives of the drugs required determining plasma concentrations over a 24 hour period, to provide accurate estimates of their area under the curve (AUCs). The half-lives of the drugs are 8hours for Pyrazinamide, 3hours for Isoniazid slow acetylators and 1hour for fast acetylators, 4hours for Ethambutol, and 3hours for Rifampicin).

The samples obtained were stored at minus 70°C for batch (blind) assaying for the anti-TB drugs. These were then transported in vapor phase liquid nitrogen (dry ice) tanks to London for analysis.
2.5 HIV COUNSELING

The chief investigator as well as local counselors, from the home-based care teams, did Pre and Post-test counseling. HIV positive patients were referred to the home-care teams as well as the social welfare department for further support.

Confidentiality of the patients’ status was maintained at the discretion of the patients. A lot of the potential patients’ were excluded because they refused to give permission for the test. This was mainly due to the social stigma associated with the disease as well as the fact that no form of direct interventional therapy (i.e. anti-retroviral drugs) was being offered due to the cost!

2.6 LABORATORY METHODS

2.6.1 BASE-LINE INVESTIGATIONS

These were performed using the standard diagnostic tests available at the UTH.

**HIV Serology:**

Plasma of the potential recruits was tested using the following antibody tests:

- Capillus® HIV-1/HIV-2 by Cambridge Diagnostics.
- Enzyme Linked Immuno-assay test using Wellcozyme® HIV recombinant test kits (manufactured by Murex Diagnostics).
- Western Blot Assay (by Genelabs Diagnostics) as a confirmatory test for HIV-1.

**Hematology:** was done using the automated Coulter T-660 machine.

Renal and Liver function tests were performed automatically using the Cobas MiraS.
Chest X-ray and direct examination of sputum using the Ziel-nielsen stain (Acid and Alcohol Fast bacilli) achieved diagnosis of Pulmonary Tuberculosis.

**T-cell subset counts** (CD4/CD8) were performed using a *Becton Dickinson's*® *Facs-count* fully automated machine. It contains antibody reagents and a quantification technique (reference beads). The Facs-count is able to analyze the following from a serum sample:

- CD4 (helper/ Inducer T-lymphocytes)
- CD8 (Suppressor/Cytotoxic T-lymphocytes)
- CD3 (Total T-lymphocytes)

The CD4 antigen is the receptor for HIV (Dalgleish, 1984). The absolute number of CD4 T-lymphocytes is the cellular parameter most closely associated with HIV disease progression and patient prognosis (Fahey, 1990).

**2.6.2 DRUG ASSAYS**

As earlier mentioned this was done at the University College London Medical School for the specified reasons.

Since combined therapy is the exclusive form of treatment of mycobacterial diseases, it was important that any analytical method be specific, with no interference from the other co-administered antimycobacterials. The High Performance Liquid Chromatography (HPLC) methods utilized in this study had this attribute. Another point of note was that the retention times of the other drugs could lead to spurious peaks, as they were not always reported. With this in mind appropriate blank serum samples and aqueous drug solutions were analyzed to correctly identify the peaks in the chromatogram.

Plasma estimations at steady state were performed for *Isoniazid, Pyrazinamide* and *Rifampicin* using the HPLC methods outlined below. As earlier mentioned analytical difficulties precluded the measurement of *Ethambutol*. Each of the methods utilized were validated before assaying of the clinical samples.
(a) ISONIAZID

Plasma total isoniazid concentrations were determined using HPLC method of Hutchings et al. (1983), that utilized a reverse phase nitrile (CN) column (Spherosorb nitrile; Anachem®, Luton, UK). The mobile phase was 0.01 mol/L phosphoric acid in acetonitrile: water (20:80 v/v) pumped at 2 ml/min and detection was by Ultra Violet (UV) absorbance at 266 nm. Calibrators were prepared using known amounts of isoniazid concentrations ranging from 0.1-15mg/L.

(b) PYRAZINAMIDE

Pyrazinamide in plasma was estimated by using the method of Chan et al.(1986). This used a reversed phase C18 column (Hibar, LiChrocart RP-8; Merck®) with a mobile phase of acetonitrile: 0.01 mol/L pH 3.5 phosphate buffer (1:9 v/v) pumped at a flow rate of 1.5 ml/min. Detection was once again by UV absorbance at 215 nm. The problem of pyrazinamide adsorbing on to the glass tube was overcome by pre-treating the glassware with a siliconizing agent. A calibration curve covering the range of 0-60mg/L was prepared to calculate the pyrazinamide concentrations.

(c) RIFAMPICIN

Plasma total Rifampicin concentrations were estimated using the HPLC method described by Ogata et al.(1988). The column used was Nucleosil C18 250 × 4.0 mm (Fisons®, Loughborough, UK) operated at 40°C and the mobile phase utilised was acetonitrile: 0.1 mol/L potassium phosphate pH 4.0 (38:62 v/v) pumped at a flow rate of 1.2 ml/min. UV absorption at 340 nm was utilised for detection. A calibration curve was constructed with serum calibrators covering the range 0.5- 25mg/L. Problem of Rifampicin adsorbing to glass or plastic was minimized by using small volumes in large containers and pre-filled pipettes to saturate any binding sites.
2.7 DATA COLLECTION AND ANALYSIS

A uniform data-recording sheet was used for all cases (HIV positive patients’ with/without diarrhea) and control groups (HIV negative with no diarrhea).

Any significant signs or symptoms were included on the data sheet as well as laboratory data such as urea, creatinine and T cell subsets.

Plasma concentration (C) versus Time (T) data were analyzed by noncompartmental methods. The highest plasma concentration observed of the drug was defined as Cmax. The area under the curve from 0 to 8-hour period was obtained using the trapezoidal rule. The 8 to 24 hours time period was estimated by applying the log trapezoidal rule, thus the area under the curve for the 24-hour period (designated AUC) was then obtained. The calculations were done using the software package Sigma Plot (Jandel ® Corporation).

Data entry and statistical calculations were then accomplished by using computer-based data management system (Epi-info) with appropriate statistical and graphics software.

To detect significant difference in the pharmacokinetic parameters (Cmax and AUC) between the three groups, analysis of variance (ANOVA) was used, with HIV status and the presence of chronic enteritis as the factors. The groups were compared using non-parametric statistics (Kruskal- Wallis H test, which is equivalent to the Chi square). A P-value less than 0.05 was considered statistically significant for all the tests.
CHAPTER 3

3 RESULTS

"A witty statesman said, you might prove anything by figures"
(Thomas Carlyle 1795-1881)

3.1 PATIENTS

Over a one-year period over 500 patients were evaluated for entry into the study. Of these sixty patients met the study criteria and were recruited into three groups of twenty. The individual demographic and clinical characteristics are shown in Table 2.

There were fewer females included in the study because of the added exclusion criteria (pregnancy and lactation) as well as the refusal by some 'partners' to undergo the serological tests.

The mean Body Mass Index (BMI= kg/m²) of all the three groups was below normal. This was expected as Tuberculosis, known as "Phthisis" (wasting) in the time of Hippocrates, itself causes weight loss. The medians of the anthropometric measurements in the three groups were not statistically significant (P <0.05). Biochemical data was not assessed due to the obvious bias associated with the study as patients were excluded if the tests were abnormal.

The CD4 counts and the CD4/CD8 ratios for the HIV negative group had few aberrations. There were five values below the 500-cells/ml mark, two of which were in the AIDS defining category level (<200-cells/ml). Reduced levels of CD4 counts have been documented in HIV negative patients with tuberculosis but not at this level! Possible causes could have been:
The two cases documented with CD4 counts <200 had BMI of 15kg/m² (this was on the lower range of the values obtained in the study). Malnutrition per se has been shown to cause depletion of the CD4 counts.

Error in the technique of measurement. The Facs-count machine was always said to have been standardized before each sample was run. Nonetheless this still remains the most likely scenario as other unpublished studies by Zumla have observed erroneous results before. This last detail does create a doubt on the validity of all the CD4 results.

With the two values at the AIDS defining level there is possibility that anergy in the patient lead to a poor antibody response which could barely be detected by the HIV serological test kits. This could have been resolved using the viral load test as evidence of HIV infection. Nonetheless this test was not available locally and the patients were not willing to have a repeat test done. The general ‘good’ condition of the patients tended to preclude this possibility.

Finally the commercial test kits used mainly detect HIV-1 subtype B antibodies. However other sub-types have been documented in the sub-region, so the possibility of false negatives is not ruled out (Kanki et al., 1997).

These values lowered the overall mean of the CD4 count in the HIV negative group, but even when the affected patients’ drug levels were discounted in the statistical analysis, no significant change in the result of the overall objective of the study was noted!

The median CD4s’ and the ranges in the HIV positive PTB patients with and without diarrhoea were statistically comparable, meaning that the CD4 count does not always correlate with the presence of chronic diarrhoea. Finally there was an obvious difference in the CD4s’ on comparing the two HIV positive groups with the HIV negative group.
Table 2. Demographic Characteristics of Study Participants

<table>
<thead>
<tr>
<th>Characteristics (Ranges)</th>
<th>PTB patients and HIV negative (Group A)</th>
<th>PTB patients and HIV positive (Group B)</th>
<th>PTB patients and HIV positive with Diarrhea (Group C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age, y</td>
<td>32.5 (16-58)</td>
<td>33 (20- 50)</td>
<td>32 (24- 51)</td>
</tr>
<tr>
<td>Median body weight, kg</td>
<td>50 (40- 65)</td>
<td>50 (40- 110)</td>
<td>49 (36- 62)</td>
</tr>
<tr>
<td>Body Mass Index, kg/m2</td>
<td>17.72 (15.05- 21.38)</td>
<td>18.44 (15.42- 38.51)</td>
<td>17.75 (14.34- 21.63)</td>
</tr>
<tr>
<td>Sex, n</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>18</td>
<td>10</td>
<td>16</td>
</tr>
<tr>
<td>Female</td>
<td>2</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>Median CD4+ cell count, cells/mm3</td>
<td>550.5 (60-1442)</td>
<td>153 (1- 879)</td>
<td>191.5 (1- 438)</td>
</tr>
<tr>
<td>Median CD4/CD8 ratio (Mean)</td>
<td>1.175 (1.280)</td>
<td>0.180 (0.340)</td>
<td>0.200 (0.320)</td>
</tr>
</tbody>
</table>

3.2 PHARMACOKINETIC ANALYSIS

All the patients received the standard doses of the antimycobacterial drugs for their specific weight (see appendix 3). Careful inquiry of the bowel motions on the study day was made. Seven of the patients in the chronic enteritis group had more than 3 loose motions the day before the study. One of the patients even had to be admitted for a further day after the study for treatment of the intractable diarrhea.

None of the three groups of patients were on any other medication besides the ATT for at least one-week prior to the admission. A few of chronic enteritis patients were on other medication on their sampling day, but this variable was taken into account and the study day postponed by a week in these patients till above condition was met.
The median mg/kg dose administered for the three groups A, B, and C respectively, were:

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoniazid</td>
<td>8.00,</td>
<td>8.00</td>
<td>6.11</td>
</tr>
<tr>
<td>Pyrazinamide</td>
<td>35.00,</td>
<td>35.00</td>
<td>30.60</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>12.00</td>
<td>12.00</td>
<td>9.18.</td>
</tr>
</tbody>
</table>

The pharmacokinetic attributes of Isoniazid, Rifampicin and Pyrazinamide in the three groups are given in tables 3, 4 and 5. The means of the pharmacokinetic profiles compared favorably to the normal values of the individual drugs (P< 0.05).

The participants were further classified as either fast or slow acetylators if they had an Isoniazid t½ of less than 130 minutes. The half-life of isoniazid was used as it has been shown to correlate well with the acetylator status of the patient (Jittinen H, 1969). There were 5, 5 and 4 fast acetylators in groups A (HIV negative), B (HIV positive without diarrhea) and C (HIV positive with diarrhea) respectively. (See figure 3). This was roughly equivalent to 23% of the study population. The peaks of the bimodal distribution of isoniazid half-life corresponded to the values in literature of fast and slow acetylators (P = 0.01).

Neither the presence of diarrhoea nor HIV accounted for the inter-patient variability in AUC or Cmax of the three drugs. There were no significant differences in the calculated Area under the Curve (AUC) and maximum measured drug concentration (Cmax) between the three groups i.e. Group A, B and C for the drugs, Isoniazid, Pyrazinamide and Rifampicin at steady-state concentrations (P > 0.05) (see Table 6). Even when the 5 patients with CD4 counts of less than 500 in group A (HIV negative) were excluded the P value (= 0.389) obtained still remained insignificant.
## Table 5

<table>
<thead>
<tr>
<th>AUC (µg/mL.min)</th>
<th>Clearance (ml/min/kg)</th>
<th>Cmax (µg/ml)</th>
<th>Vd (l/kg)</th>
<th>t1/2 hours</th>
</tr>
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<tbody>
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<td>A</td>
<td>B</td>
<td>C</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
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<td>1640.0</td>
<td>1007.0</td>
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<td>3.5</td>
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<td>940.0</td>
<td>1603.0</td>
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<td>4.2</td>
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<td>4.4</td>
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<td>4.3</td>
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<td><strong>mean</strong></td>
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<td><strong>1223.4</strong></td>
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<td><strong>SD</strong></td>
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<td><strong>441.8</strong></td>
<td><strong>2.1</strong></td>
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<td><strong>median</strong></td>
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<td><strong>1348.2</strong></td>
<td><strong>5.4</strong></td>
<td><strong>4.4</strong></td>
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</tbody>
</table>

**Normal values:**
- AUC: area under the curve
- Cmax: Maximum concentration of curve
- Vd: Volume of Distribution
- t1/2: Half-life
- SD: Standard Deviation

- Fast acetylators = 1.1 +/- 0.2
- Slow acetylators = 3.1 +/- 1.1
<table>
<thead>
<tr>
<th>Table 4</th>
<th>Pyrazinamide</th>
</tr>
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<tr>
<td><strong>AUC (µg/ml.min)</strong></td>
<td><strong>Clearance (ml/min/kg)</strong></td>
</tr>
<tr>
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<td>B</td>
</tr>
<tr>
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</table>

Mean 
SD 
Median 
Normal values 
AUC= area under the curve 
Cmax= Maximum concentration of curve 
Vd= Volume of Distribution 
t1/2= Half-life 
SD= standard deviation
### Table 3

**Rifampicin**

<table>
<thead>
<tr>
<th>AUC (ug/ml.min)</th>
<th>Clearance (ml/min/kg)</th>
<th>Cmax (ug/ml)</th>
<th>Vd (l/kg)</th>
<th>t1/2 hours</th>
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<tr>
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<td>A</td>
<td>B</td>
<td>C</td>
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</table>

**AUC** = area under the curve  
**Cmax** = Maximum concentration of curve  
**Vd** = Volume of Distribution
Figure 3. Bimodal distribution of ISONIAZID half-lives in Zambian patients

Number of subjects

Half-life, minutes (see legend)

01/01/00

half-life, minutes 0-30
half-life, minutes 30-60
half-life, minutes 60-90
half-life, minutes 90-120
half-life, minutes 120-150
half-life, minutes 150-180
half-life, minutes 180-210
half-life, minutes 210-240
half-life, minutes 240-270
half-life, minutes 270-300
half-life, minutes 300-330
half-life, minutes 330-360
half-life, minutes >360
Table 6. Mean Pharmacokinetic Variables of *isoniazid*, *rifampicin*, and *pyrazinamide*, according to the presence or absence of HIV Infection and Chronic Diarrhea.

<table>
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<tr>
<th>DRUG</th>
<th>Patient groups</th>
<th>AUC ug/ml.min</th>
<th>P-value</th>
<th>Cmax ug/ml</th>
<th>P-value</th>
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<td>Groups A and B</td>
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<td>0.383</td>
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<td>1323.4</td>
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</tbody>
</table>

Key for TABLE 2.
AUC= area under the concentration versus time curve. Cmax= maximum drug concentration on the curve. A= HIV negative without diarrhea. B= HIV positive without diarrhea. & C= HIV positive with diarrhea.
CHAPTER 4

4.1 DISCUSSION

"Let the long contention cease!
Geese are swans, and swans are geese."
(Mathew Arnold, 1822-1888)

Tuberculosis requires therapy by regimens consisting of a number of drugs that are sensitive to the particular bacilli. The purpose of therapeutic drug monitoring (TDM) is to optimize pharmacotherapy. By adjusting doses on basis of serum drug concentrations, one is able to elicit the desired therapeutic response while minimizing dose related drug toxicity. However, as high cure rates have been achieved historically with standard doses of the first-line anti-tuberculosis drugs (isoniazid, rifampicin, pyrazinamide and ethambutol), there has been limited interest in applying TDM as a therapeutic tool till now (Peloquin et al., 1993).

Response to anti-mycobacterial therapy in HIV positive patients is generally good and comparable to the non-HIV infected patients (Chaisson et al., 1987: Alwood K, 1994), though the latter have been associated with a higher relapse rate.

Nonetheless, the recent resurgence of Tuberculosis with the disconcerting reports of low therapeutic serum levels in AIDS patients has made TDM more attractive at present time. The hypothesis being that AIDS, through it’s gastrointestinal associations such as gastric hypoacidity, enteropathy and opportunistic bowel infections may
predispose to the malabsorption of antituberculosis drugs (Patel et al., 1995).

The consequences of this being treatment failure in the patient and a far worse scenario of acquired multi drug resistance tuberculosis (MDR-TB) bacilli being unleashed to the general public. This is due to the fact that malabsorption of part of the treatment could potentially expose the multiplying bacilli to monotherapy, leading to the resultant overgrowth of drug resistant mycobacterial bacilli. There have already been unpublished reports in the Chest Clinic (UTH, Lusaka) that the number Multi-Drug Resistant Tuberculosis cases are on the increase.

Although there is previous documentation showing depressed serum concentrations of antituberculosis drugs in HIV/TB co-infected patients especially those with severe immunosuppression, the call to introduce screening of the drug levels, as a useful addition in these patients is still too early. This is because extensive research has shown that monitoring clinical response is still the most practical course.

Human pharmacokinetic studies provide direct, straightforward information, particularly in the four major anti-tuberculosis drugs whose vital role in the chemotherapy is clearly established. It is well known that the rate and extent of the bioavailability of the drug is reflected in the concentration of the active ingredient appearing in the blood at various points after administration.

It would have been ideal to measure the bioavailability of the drugs singularly to prevent interference from the associated drugs but this would have been at the detriment of the patient (monotherapy is not recommended) and the community (increased drug resistance).

In this study, the pharmacokinetic parameters at steady-state of orally administered Isoniazid, Rifampicin, and Pyrazinamide were measured in 60 PTB patients:
- 20 of whom were infected with HIV and had history of chronic enteritis (Group C)
• 20 HIV positive patients without history of diarrhoea (Group B),
• 20 HIV negative patients (who served as a control group) (Group A).
Ethambutol was administered to the patients but not assayed due to technical difficulties (drug assay method had not been validated in time).

The mean pharmacokinetic profiles of the three drugs in the control group compared favorably to the normal parameters documented in literature (see Table 1). When variations in acetylation were taken into account, even the half-lives of Isoniazid measured up to the expected normal values.

The acetylator status of the patients was also defined. This was important as the rate of metabolism (acetylation in the case of Isoniazid) significantly alters the concentrations of the drug in plasma and it’s half-life in circulation. The frequency of acetylation phenotype is dependent on race but not on sex or age. Fast acetylators are found in Inuit and the Japanese. Slow acetylators are predominantly found in North African Caucasians, Jews and Scandinavians. An analysis of various racial groups in the USA showed the incidence of fast acetylators at approximately 50% (Mandell et al., 1996). The incidence of fast acetylators in this study population of Zambian PTB patients was found to be 23%.

This research in Zambian PTB patients documented no evidence of impairment in the steady-state pharmacokinetics of the antituberculosis drugs between the three groups. That is neither the HIV status nor the additional presence of overt, chronic enteritis affected the pharmacokinetic profile of *Isoniazid*, *Rifampicin*, and *Pyrazinamide*. The main parameters tested were the area-under the concentration versus time curve (AUC), and the maximum measured concentration (C-max). These included the HIV positive patients with reported overt enteritis.

All the peak concentrations observed in this study were well above the minimum inhibitory concentrations for sensitive organisms: 0.025- 0.05 ug/ml for *Isoniazid*, 0.005- 0.2 ug/ml for *Rifampicin*, and 12.5 ug/ml for bactericidal concentrations of *Pyrazinamide* (Heifets, 1991). However, it should be noted that the serum level of the drug does not necessarily correlate with adequate antituberculous activity in the infected tissue!
The low drug levels specifically attributable to HIV related processes, reported in the literature is questionable, as it has mainly been documented using single serum measurements. It is more important to assess the pharmacological profile over 12-24-hour time period and not just take single random samples. This could lead to erroneous documentation of low serum levels of anti-tuberculosis therapy.

The lack of observing any effect of HIV and diarrhoea on the pharmacokinetics of the anti-tuberculosis drugs could have been because of the small number of patients. However the sample size was calculated to detect a significant difference in the drug absorption (at the 5% level i.e. > 2 standard deviation from 'normal'). However it is still possible that small differences (less than the above figure) could have occurred as had been so ably documented in the previous studies (Peloquin et al., 1996; Choudri et al., 1997). Finally, it should still be noted that a substantial reduction in total drug exposure during the course of therapy could still have profound clinical consequences, especially, if this reduction is combined with pre-existing drug heteroresistance, advanced immunodeficiency, and reduced compliance.
4.2 CONCLUSION

"Life is the art of drawing sufficient conclusions from insufficient premises"
(Samuel Butler, 1835-1902)

This study did not demonstrate any momentous contributions of HIV infection, especially associated overt diarrhoea disease to the variable pharmacokinetics in PTB patients in Lusaka, Zambia.

Till further clarification is determined concerning the particular aspects of the pharmacological profile of antituberculosis drugs linked to the therapeutic outcome (both in HIV positive and negative patients), therapeutic drug monitoring based on one or two blood samples should not be generally regarded as a clinically useful tool in either population. Sequential monitoring of the drug levels should be determined.
4.3 STUDY LIMITATIONS

A few limitations were noted in the study. These included the following:

1. The bowel motion status was dependent on verbal confirmation by the patients in the study. Furthermore the presence of malabsorption was not confirmed. The addition of the D-xylose test (a relatively specific measure of proximal small-bowel absorption) would have added strength to the results of the study.

2. The sample size comprised only 60 patients. Nevertheless as this was a pharmacological study requiring intensive monitoring/sampling of the patients, the results obtained are still considered significant. The sample size was extrapolated by log transformation from another study with a view to detect a significant drop in the serum levels of more than 1 standard deviation (>66.66%) from the control.

3. A combination drug, Rifinah® (combination of Isoniazid and Rifampicin) instead of separate drugs. However previous studies concerning the bioavailability of combination ATT drugs showed no difference when compared to single drugs used in the regimens. In addition all three groups received the same drugs, thus had the same variable.

4. The distance between the study site and the drug-assay center presented a few logistical problems. Future pharmacological studies should consider setting up on-site assaying facilities.

5. The discrepancies concerning the CD4 counts have already been discussed above.
4.4 RECOMMENDATIONS

“One gives nothing so freely as advice”
(Duc de la Rochefoucauld, 1693-1680)

- As no significant differences were noted in the pharmacokinetics between the three groups, future studies should concentrate on patients with aberrations in the monitored clinical response e.g. emergence of drug resistant strains or early onset of relapse.

- A better understanding is also required of the pharmacological parameters that significantly predict the therapeutic outcome e.g. does the duration the drug’s plasma concentration exceeds the MIC (for Mycobacterium tuberculosis) correlate with the treatment outcome (i.e. early response to therapy, the emergence of drug-resistant organisms and relapse of disease).

- Finally if low serum levels are obtained in future, it would more appropriate to do serum sampling over 12-24 hours rather than extrapolate data from a single specimen obtained.
Bibliography


Appendices

Appendix 1

Patient Information Sheet

Thank you for participating in this study that will determine whether PTB patients on treatment with diarrhea are able to absorb the drugs in adequate amounts.

You will be admitted to a sideward in E-block for two nights. Food and drink will be provided.

Please ensure that you take your anti-TB medication in the morning of the day of admission (day 1). You will be admitted to the ward at 16:00hours and some blood will be collected for some basic tests. Please do NOT EAT or DRINK anything after midnight.

On the day of the study (day 2) DO NOT TAKE THE ANTI-TB DRUGS until the doctor gives them to you..

Blood samples 8-10ml per drug analyses will be collected at the following times:
- Before the drugs are taken
- Next samples will be collected at 1,2,4,6,8 and 24hour interval after the drugs are taken.

After the last blood samples are taken the next morning (day 3) you will be provided with more of the TB drugs to ingest. You will be discharged from the ward with some haematinics and transport money will be provided.
Appendix 2

Patient Consent Form:

I __________________________

Have understood the objective of the study, which has been explained to me by Dr. S. Lakhi. The explanation included:
a) Verbal description of study and information sheet.
b) Study requirements including purpose and length of study.

I further understand that I am free to withdraw from the study at any time after giving consent without having to give a reason, and if I do this it will not affect my treatment in any way. I am also aware that competent authorized persons may scrutinize my personal information but it will be treated under strictest confidence and will not be publicly available. I consent to participate in the study in the knowledge that I will not receive any direct benefit from participating in the study.

Dated this ____________________ day of ____________________ 199___

Signed: _______________________

Witness: ______________________

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Appendix 3

Patient Form

Name: _______________  TB No: _____  Study No: _____

Date of Birth: _____________  Age (if no doubt): _____  Sex _____

Height: _____________(m)  Weight: ______________(kg)

Day 1
Date admitted: __/__/____

Last anti-TB drugs taken: __/__/____  Time: _________ hours

Day 2

First Blood sample  (8-10 ml) Time 0=_______hrs  (Now enter scheduled times).

Time anti-TB drugs given: ________ hours.  Given by: ________

Drug Dosage Chart

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<th>&gt;= 50kg</th>
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<td>4</td>
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<tr>
<td>Pyrazinamide (0.5g Tablets)</td>
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<tr>
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<tr>
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<td>Actual Time</td>
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</tr>
<tr>
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<td>-------------</td>
<td>------------</td>
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<tr>
<td>1 hr__________</td>
<td>___________ hrs</td>
<td>________</td>
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<tr>
<td>24 hr__________</td>
<td>___________ hrs</td>
<td>________</td>
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</tr>
</tbody>
</table>

**Patient Clinical Details:**
Appendix 4

HIV Test/ CD4 Count/ Urea & Creatinine/ Electrolytes/ FBC/ LFTs

Full Name: ___________________ Study No. ________

Notification No. _____________

The following specimen is forwarded to the laboratory for HIV test/ CD4 Count/ Urea & Creatinine/ Electrolytes/ FBC/ LFTs.

Date of Specimen: ___/___/___

Signature: ____________________